and one with benign insulinoma) have been operated on following this technique. Among these cases, there was 0% morbidity including splenic volvulus and pancreatic fistula.

Discussion

Open pancreatic surgery requires a relatively large incision for a small lesion, and therefore the potential benefits of the laparoscopic approach are substantial. The most common indications for laparoscopic pancreatic resection are presumed benign pancreatic diseases, such as insulinoma or localized neuroendocrine neoplasms and branch type intraductal papillary mucinous neoplasms. The most common indication for laparoscopic pancreatic resection appears to be enucleations and distal pancreatectomy.

The successful management of the pancreatic stump remains the challenge of this procedure. In some laparoscopic enucleation studies, the rate for low volume pancreatic fistula is reported to be high [9]. This complication does not create an important problem as long as the main duct is not injured. Even though self-limiting, the pancreatic fistula formation rate remains high after either laparoscopic enucleation or resection. Pancreatic fistula after distal pancreatectomy has been a concern for decades, even in the era of laparoscopic pancreatectomy. Patterson et al. collected data from the literature on morbidity after open and laparoscopic pancreatic resections, and found that the rate of pancreatic fistula ranged from 20 to 33% after laparoscopic pancreatectomy and from 5 to 23% after open pancreatectomy [10]. The way in which the surgeon approaches the pancreatic transection seems to be important. Ninety-seven percent of the patients underwent laparoscopic transection of the pancreas by use of a stapling technique [9]. Closing the pancreatic stump with interrupted mattress sutures and selectively ligating the pancreatic duct, the usual practice in open surgery, are more difficult to replicate laproscopically. This factor could explain the high rate of pancreas-related complications. Hand-sewn parenchymal closure and duct ligation are an advantage of this extracorporeal pancreatic resection, to prevent pancreatic juice leakage, compared with the procedure done by laparoscopy only. We could safely and securely handle the pancreatic duct and fine branches of the splenic vessels under direct vision.

Distal pancreatectomy with preservation of the spleen was first reported in 1988 [11]. The advantage of preserving the spleen is obvious; it reduces the risk of postoperative severe inflammation and peripheral blood count aberration. Preserving the spleen has been a major procedure in distal pancreatectomy. Warshow reported a case of splenic abscess that occurred after sacrificing the splenic artery and vein [11]. Kimura et al. reported five patients successfully treated with splenic vessel-preserving distal pancreatectomy to maintain the blood supply to the spleen and to avoid splenic necrosis and abscess [12]. Spleen-preserving pancreatectomy has recently been shown to have a risk of complication comparable to that of standard pancreatectomy where the spleen is removed. Nevertheless, spleen-preserving pancreatectomy remains an uncommon and technically demanding operation, due to the difficulty of dissecting the distal pancreas from the splenic vessels. An advantage of our procedure is the safety it provides in dissecting the distal pancreas from the splenic vessels. The displacement of the spleen with the inherent risk of torsion or hemorrhage is another disadvantage of spleen-preserving pancreatectomy. If spleen-preserving pancreatectomy is performed, the spleen is often free in the abdomen, where it is prone to torsion or trauma. Various techniques have been described to reposition the spleen (splenopexy). Appu et al. [13] report a novel technique for splenic repositioning and fixation, using a peritoneal pocket. We experienced one case of splenic bleeding due to venous congestion after spleen-preserving pancreatic tail resection using Appu's splenopexy. Since that experience we have chosen to preserve the gastrosplenic ligament.

This approach is suitable for the very distal lesion of the pancreas. However, if the posterior plane of Gerota's fascia is dissected, this method could be applied to more proximal lesions as shown in Fig. 2. For obese patients, because pulling the distal pancreas and spleen out through a small laparotomy is difficult, a 10 cm incision is preferable. This procedure is applicable only for lesions in the pancreatic body and tail. For the benign head lesions, another approach should be conducted [14].

Preservation of the gastrosplenic ligament and extracorporeal preparation of the transected pancreatic stump and splenic vessels under direct vision are useful measures in spleen-preserving distal pancreatectomy under a minimal incision approach assisted by laparoscopy.

References

1. Gagner M, Pomp A: laparoscopic pylorus-preserving pancreatoduodenectomy. Surg Endosc. 1994;8:408–10.
2. Gagner M, Pomp A, Herrera MF. Early experience with laparoscopic resections of islet cell tumors. Surgery. 1996;120:1051–4.
3. Dexter SP, Martin IG, Leindler L, Fowler R, McMahon MJ. Laparoscopic enucleation of a solitary pancreatic insulinoma. Surg Endosc. 1999;13:406–8.
4. Sussman LA, Christie R, Whittle DE. Laparoscopic excision of distal pancreas including insulinoma. Aust NZ J Surg. 1996;66:414–6.
5. Hama T, Takifuji K, Uchiyama K, Tani M, Kawai M, Yamaue H. Laparoscopic splenectomy is a safe and effective procedure for patients with splenomegaly due to portal hypertension. J Hepatobiliary Pancreat Surg. 2008;15:304–9.

6. Klingler PJ, Hinder RA, Menke DM, Smith SL. Hand-assisted laparoscopic distal pancreatectomy for pancreatic cystadenoma. Surg Laparosc Endosc. 1998;8:180–4.
7. Shinchi H, Takao S, Noma H, Mataki Y, Iino S, Aikou T. Hand-assisted laparoscopic distal pancreatectomy with minilaparotomy for distal pancreatic cystadenoma. Surg Laparosc Endosc Percutan Tech. 2001;11:139–43.
8. Kaneko H, Takagi S, Joubara N, Yamazaki K, Kubota Y, Tsuchiya M, et al. Laparoscopy-assisted spleen-preserving distal pancreatectomy with conservation of the splenic artery and vein. J Hepatobiliary Pancreat Surg. 2004;11:397–401.
9. Mabrut JY, Fernandez-Cruz L, Azagra JS, Bassi C, Delvaux G, Weerts J, et al. Laparoscopic pancreatic resection: results of a multicenter European study of 127 patients. Surgery. 2005;137:597–605.
10. Patterson EJ, Gagner M, Salky B, Inabnet WB, Brower S, Edye M, et al. Laparoscopic pancreatic resection: single-institution experience of 19 patients. J Am Coll Surg. 2001;193:281–7.
11. Warshow AL. Conservation of the spleen with distal pancreatectomy. Arch Surg. 1988;123:550–3.
12. Kimura W, Inoue T, Futakawa N, Shinkai H, Han I, Muto T. Spleen-preserving pancreatectomy with conservation of the splenic artery and vein. Surgery. 1996;120:885–90.
13. Appu S, Young AB, Lawrentschuk N. Peritoneal "pillowcase" for the displaced spleen post-distal pancreatectomy. J Hepatobiliary Pancreat Surg. 2005;12:470–3.
14. Hirota M, Kanemitsu K, Takamori H, Chikamoto A, Ohkuma T, Komori H, et al. Local pancreatic resection with preoperative endoscopic transpapillary stenting. Am J Surg. 2007;194:308–10.

Whole Stomach and Spleen Preserving Total Pancreatectomy: A New Surgical Technique for Pancreatic Cancer

Michiko Harao[1,2], Shoichi Hishinuma[1], Morihiro Tomikawa[1], Hideo Baba[2], Yoshiro Ogata[1]
[1]Department of Surgery, Tochigi cancer center, Utsunomiya, Japan
[2]Department of Gastroenterological Surgery, Graduate School of Kumamoto University, Kumamoto, Japan
Corresponding Author: Michiko Harao, MD,PhD
Department of Surgery, Tochigi cancer center, Utsunomiya, Japan
Tel: +81286585151, Fax: +81286585669, E-mail: miharao@tcc.pref.tochigi.lg.jp

SUMMARY

Total pancreatectomy has been used to treat both benign and malignant diseases of the pancreas. The procedure of total pancreatectomy for invasive pancreatic cancer usually includes distal gastrectomy and splenectomy to prevent ischemic changes due to decreased blood supply. In this report, it was introduced a new technique of total pancreatectomy for invasive pancreatic cancer preserving both the whole stomach and spleen. The patient was a 61 year old man. Preoperative computed tomography (CT) showed a mass of tumor, measuring 23×18×25mm, located in the pancreatic head. It was tried, initially to perform pylorus-preserving pancreatoduodenectomy (PPPD). Repeated frozen section examination of the pancreatic stumps, however, revealed persistent cancer infiltration to the distal pancreas. Therefore, we altered the planned PPPD to total pancreatectomy preserving the whole stomach and spleen with severing both the splenic artery and vein at their origins. The postoperative course was uneventful. Enhanced CT following surgery showed sufficient blood supply to the whole stomach and spleen without any congestive changes of blood flow. This method is considered safe and useful for patients with both benign and malignant disease of the pancreas.

KEY WORDS: Total pancreatectomy; Pancreatic cancer; Preservation of the spleen; Preservation of the stomach

ABBREVIATIONS: Computed Tomography (CT); Gastroduodenal Artery (GDA); Left Epigastric Artery (LEGA); Left Gastric Artery (LGA); Pancreatoduodenectomy (PPPD); Right Epigastric Artery (REGA); Right Gastric Artery (RGA); Splenic Artery (SpA); Splenic Vvein (SpV)

INTRODUCTION

Total pancreatectomy for invasive pancreatic cancer usually includes distal gastrectomy and splenectomy to prevent ischemic changes due to decreased blood supply. Most reports of spleen preservation in pancreatic resection relate to distal pancreatectomy (1, 2, 3).[1]In this report, we introduce a new technique of total pancreatectomy for invasive pancreatic cancer preserving both the whole stomach and spleen.

CASE REPORT

A 61-year-old man was admitted to Tochigi Cancer Center Hospital with a complaint of epigastralgia. Laboratory results on admission were as follows : leukocyte count, 6380cells/mm^3 ; hemoglobin, 13.3g/dl; platelet count, 269000cells/mm^3; asparate aminotransferase, 15IU/L; alanine aminotransferase, 17IU/L; alkaline phosphatase, 303IU/L; carbohydrate antigen, 17U/ml; FBS 114mg/dl and HbA1c 5.3%. Laboratory tests for tumor markers revealed a carcinoembryonic antigen value of 1.7ng/ml, and a carbohydrate antigen 19-9 value of 61.5U/ml.

Ultrasonography revealed a hypoechoic mass of tumor in the head of the pancreas, measuring 23×18×25mm. The main pancreatic duct distal to the tumor was dilated to about 10mm in diameter. Helical dynamic CT revealed a poorly enhanced tumor with pooling of contrast medium in the delayed phase. There were no signs of distant and regional lymph node metastases. Endoscopic retrograde pancreatography showed the main pancreatic duct was interrupted 2 cm distal to the papilla vater. With a preoperative diagnosis of cancer of the pancreatic head, PPPD was planned.

At laparotomy, there was no evidence of peritoneal dissemination or liver metastasis. Following mobilization of the duodenum and pancreatic head, the right gastroepiproic and right gastric vessels were severed at the pylorus. The duodenum was divided 2cm distal to the pylorus. The pancreas was then divided at the left side of the portal vein. The cut edge of the proximal pancreas was submitted to frozen section examination and found to have cancer cells. Three additional cut surfaces of the distal pancreas were also found to have cancer cells by frozen section examination. Hence, we abandoned preservation of the distal pancreas and decided to perform total pancreatectomy. The splenic artery was divided at its origin and the splenic vein was divided at the confluence with the superior mesenteric vein. The distal portion of the splenic artery and vein were severed at the hilum of the spleen without retraction of the spleen from the retroperitoneum in order not to sacrifice retroperitoneal drainage veins of the spleen. The left gastric vein had already been cut during dissection of the

Hepato-Gastroenterology 2009; 56:1549-1551

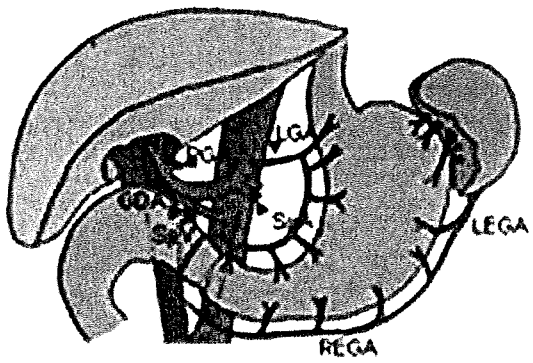

FIGURE 1
Removed part of the duodenum and total pancreas, splenic artery and vein. LGA: left gastric artery, RGA: right gastric artery, GDA: gastroduodenal artery, SpV: splenic vein, SpA: splenic artery, LEGA: left epigastric artery, REGA: right epigastric artery

lymph nodes around the common hepatic artery. The short gastric vessels were carefully preserved. Then, the blood supply to the whole stomach and spleen was only via the left gastric artery and their venous drainage was via the esophagus and retroperitoneum (**Figure 1**).

After confirming neither ischemia nor venous congestion of the whole stomach with preserved 1cm-long duodenum and spleen, gastrointestinal continuity was restored by end-to-end duodenojejunostomy and end-to-side choledochojejunostomy.

The postoperative course of this patient was uneventful. After 6 months from surgery, enhanced CT showed sufficient blood supply to the stomach and spleen without splenomegaly (**Figure 2**). Endoscopic examination revealed neither esophageal nor gastric varices. The blood sugar level was well controlled with routine self injection of insulin and hypoglycemic attack rarely occurred. This patient was able to maintain a good quality of life and accomplish planned adjuvant chemoradiotherapy. He died of liver metastasis 3 years after surgery without local recurrence.

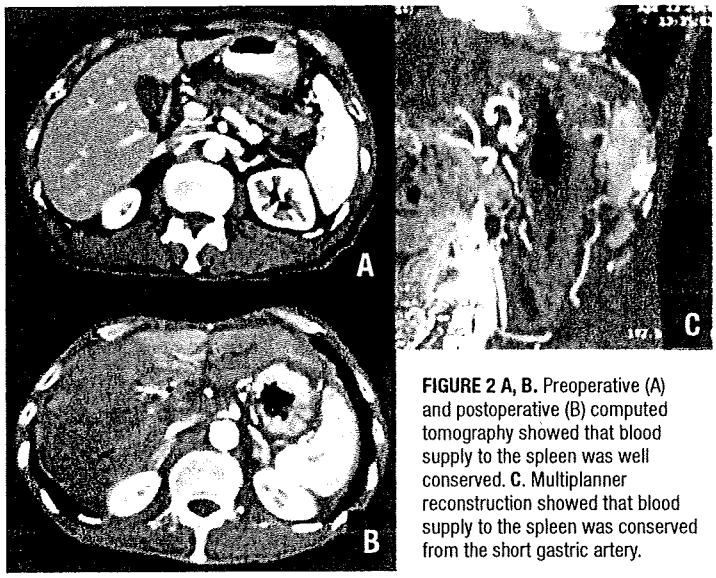

FIGURE 2 A, B. Preoperative (A) and postoperative (B) computed tomography showed that blood supply to the spleen was well conserved. **C.** Multiplanner reconstruction showed that blood supply to the spleen was conserved from the short gastric artery.

DISCUSSION

The significance of preservation of the spleen has been widely recognized, because it became apparent that severe inflammation often occurs after splenectomy in both children and adults. (1,4-6). Another advantage of spleen preservation is that a peripheral blood count keeps normal lesion, especially in the white cell or platelet counts (1,2). Furthermore, individuals splenectomized in conjunction with surgery for non-malignant conditions of adjacent organs had significant increases of lung and ovarian cancers (7). The spleen, potentially having both metabolic and immunologic functions, is worthy of being preserved if safely achieved (8). Most reports of spleen preservation in pancreatic resection relate to distal pancreatectomy (1, 2, 3), and several different variations have been described. Warshaw *et al.* (2) first reported on the feasibility of distal pancreatectomy with spleen preservation. The spleen was preserved with the short gastric vessels, while the splenic artery and vein were divided 2cm distal from the splenic hilum so as to preserve the left gastroepiploic artery and vein (2). White *et al.* (9) also documented that splenic preservation was feasible with intact short gastric and gastroepiploic vessels. Kimura *et al* (1) performed distal pancreatectomy with preservation of the splenic artery and vein, which aimed to secure blood flow of the spleen.

During total pancreatectomy for pancreatic cancer, the distal portion of the stomach is usually removed en bloc because of its possible ischemic change. In this case, the whole stomach was successfully preserved without any postoperative complications. It is well known that patients undergoing distal gastrectomy often suffer from poorly-controlled glycemia, in particular from hypoglycemia (10). Therefore, it seems more difficult to keep well-controlled glycemic conditions after total pancreatectomy when distal gastrectomy is combined. In this case, preservation of the whole stomach undoubtedly contributed to maintenance of well-controlled blood sugar level and quality of life of this patient.

In patients with pancreatic cancer, total pancreatectomy is performed if a pancreatic head carcinoma reaches too close to the plane of a possible Whipple resection, or if a carcinoma of the body or tail of the pancreas reaches too far to the right, i.e. into the pancreatic head (10). Generally total pancreatectomy involves en bloc resection of the pancreas, duodenum, spleen, and distal half of the stomach, in view of blood supply and drainage of those organs. In the present case, total pancreatectomy was carried out preserving the spleen, and whole stomach with sacrificing the splenic artery and vein. The potential complications related to this operative procedure are ischemia or blood congestion of the stomach and spleen. Ischemia of the stomach will induce anastomotic leakage of duodenojejunostomy. Blood congestion of the stomach and spleen may provoke gastric varices and severe splenomegaly. During the 3 years of survival, these episodes were not observed in this patient. Pres-

ervation of the spleen without retraction from the retroperitoneum may contribute to development of venous drainage pathways of the stomach and spleen.

To evaluate the long-term outcome of this procedure, further accumulation of case numbers is necessary. However, this study suggests that this procedure is feasible and can be adopted for patients not only with invasive ductal carcinoma of the head of the pancreas, but also with pancreatic trauma, chronic or acute pancreatitis, benign or malignant tumor of the pancreas.

REFERENCES

1. **Kimura W, Inoue T, Futakawa N, Shinkai H, Han I, Muto T:** Spleen-preserving distal pancreatectomy with conservation of the splenic artery and vein. Surgery 1996;120:885-890.
2. **Warshaw AL:** Conservation of the spleen with distal pancreatectomy. Arch Surg 1988;123:550-553.
3. **Benoist S, Dugue L, Sauvanet A, Valverde A, Mauvais F, Paye F, Farges O, Belghiti J:** Is there a role of preservation of the spleen in distal pancreatectomy? J Am Coll Surg 1999;188:255-260.
4. **Leonard AS, Giebink GS, Baesl TJ, Krivit W:** The overwhelming postsplenectomy sepsis problem. World J Surg 1980;4:423-432.
5. **Francke EL, Neu HC:** Postsplenectomy infection. Surg Clin North Am 1981;61:135-155.
6. **Sugimachi K, Kodama Y, Kumashiro R, Kanematsu T, Noda S, Inokuchi K:** Critical evaluation of prophylactic splenectomy in total gastrectomy for the stomach cancer. Gann 1980;71:704-709.
7. **Linet MS, Nyren O, Gridley G, Mellemkjaer L, McLaughlin JK, Olsen JH, Adami HO, Fraumeni JF, Jr.:** Risk of cancer following splenectomy. Int J Cancer 1996;66:611-616.
8. **Fatouros M, Bourantas K, Bairaktari E, Elisaf M, Tsolas O, Cassioumis D:** Role of the spleen in lipid metabolism. Br J Surg 1995;82:1675-1677.
9. **White SA, Sutton CD, Weymss-Holden S, Berry DP, Pollard C, Rees Y, Dennison AR:** The feasibility of spleen-preserving pancreatectomy for end-stage chronic pancreatitis. Am J Surg 2000;179:294-297.
10. **Michael Trede, David C. Carter, Churchill Livingstone:** Surgery of the pancreas 1993: 447-464

Serine Protease Inhibitor Kazal Type 1 (SPINK1): Beyond the Trypsin Inhibitor

Masaki Ohmuraya[1,2,3], Nobuyuki Ozaki[1,3], Masahiko Hirota[4], Hideo Baba[3] and Ken-ichi Yamamura*,[1]

[1]Division of Developmental Genetics, Institute of Molecular Embryology and Genetics; [2]Priority Organization for Innovation and Excellence; [3]Department of Gastroenterological Surgery, Kumamoto University, Kumamoto 860-0811, Japan and [4]Department of Surgery, Kumamoto Regional Medical Center, Kumamoto, 860-0811, Japan

Abstract: Serine protease inhibitor Kazal type 1 (SPINK1) was originally identified as a trypsin inhibitor in the pancreatic acinar cells in 1948. Recent studies showed an association of mutations in SPINK1 gene and hereditary chronic pancreatitis. Thus, a lack of SPINK1 may result in the premature conversion of trypsinogen into active trypsin in acinar cells, leading to pancreatitis. However, we found that mice deficient for Spink3, a mouse homologue of SPINK1, died after birth due to excessive autophagy (cellular self-digestion) in the pancreatic acinar cells, suggesting that Spink3 is involved in the regulation of autophagy. We further demonstrated that autophagy is involved in trypsinogen activation within the pancreatic acinar cells in experimentally induced pancreatitis. These results suggest that Spink3 has protective roles in pancreatitis by dual mechanisms, one as a trypsin inhibitor and a second as a suppressor of trypsinogen activation through negative regulation of autophagy. On the other hand, SPINK1 is structurally similar to epidermal growth factor (EGF), in terms of the number of amino acid residues and the presence of 3 intrachain disulfide bridges. In fact, Spink3 acts as a growth factor in various cell lines *in vitro*. To gain additional insight into the new function of Spink3 *in vivo*, we examined the expression pattern of Spink3 during development. We found that Spink3 was expressed in unexpected tissues such brain and mesonephric tubules. In this review, we summarize the old and new roles of SPINK1/Spink3 in trypsin inhibition, autophagy, and cell proliferation/differentiation.

Keywords: SPINK1, Spink3, PSTI, autophagy, EGF, EGFR, seminal vesicle.

Current Enzyme Inhibition, 2009, 5, 110-116

Serine Protease Inhibitor Kazal Type 1 (SPINK1): Beyond the Trypsin Inhibitor

Masaki Ohmuraya[1,2,3], Nobuyuki Ozaki[1,3], Masahiko Hirota[4], Hideo Baba[3] and Ken-ichi Yamamura*,[1]

[1]*Division of Developmental Genetics, Institute of Molecular Embryology and Genetics;* [2]*Priority Organization for Innovation and Excellence;* [3]*Department of Gastroenterological Surgery, Kumamoto University, Kumamoto 860-0811, Japan and* [4]*Department of Surgery, Kumamoto Regional Medical Center, Kumamoto, 860-0811, Japan*

Abstract: Serine protease inhibitor Kazal type 1 (SPINK1) was originally identified as a trypsin inhibitor in the pancreatic acinar cells in 1948. Recent studies showed an association of mutations in SPINK1 gene and hereditary chronic pancreatitis. Thus, a lack of SPINK1 may result in the premature conversion of trypsinogen into active trypsin in acinar cells, leading to pancreatitis. However, we found that mice deficient for Spink3, a mouse homologue of SPINK1, died after birth due to excessive autophagy (cellular self-digestion) in the pancreatic acinar cells, suggesting that Spink3 is involved in the regulation of autophagy. We further demonstrated that autophagy is involved in trypsinogen activation within the pancreatic acinar cells in experimentally induced pancreatitis. These results suggest that Spink3 has protective roles in pancreatitis by dual mechanisms, one as a trypsin inhibitor and a second as a suppressor of trypsinogen activation through negative regulation of autophagy. On the other hand, SPINK1 is structurally similar to epidermal growth factor (EGF), in terms of the number of amino acid residues and the presence of 3 intrachain disulfide bridges. In fact, Spink3 acts as a growth factor in various cell lines *in vitro*. To gain additional insight into the new function of Spink3 *in vivo*, we examined the expression pattern of Spink3 during development. We found that Spink3 was expressed in unexpected tissues such brain and mesonephric tubules. In this review, we summarize the old and new roles of SPINK1/Spink3 in trypsin inhibition, autophagy, and cell proliferation/differentiation.

Keywords: SPINK1, Spink3, PSTI, autophagy, EGF, EGFR, seminal vesicle.

There are two types of trypsin inhibitors in the pancreas, basic pancreatic trypsin inhibitor (BPTI), and pancreatic secretory trypsin inhibitor (PSTI) [1]. BPTI strongly inhibits trypsin, chymotrypsin, plasmin and kallikrein, but is not secreted into pancreatic juice. The human pancreas, however, does not have BPTI.

Kazal *et al.* purified bovine PSTI from a side fraction of a commercial insulin process in 1948 [2]. PSTI can be distinguished from the BPTI on the basis of its lack of inhibitory effect on chymotrypsin and pancreatic kallikrein [3]. The gene encoding human or mouse PSTI has been recently named the serine protease inhibitor, Kazal type 1 (SPINK1), or Spink3, respectively. The SPINK1 gene is located on chromosome 5 and is composed of four exons, spanning about 7.2 kb in length [4]. The SPINK1 gene encodes an mRNA of 237bp, which is translated to a 79 amino acid peptide including a 23 amino acid signal peptide. The secreted SPINK1 consists of 56 amino acids with three intramolecular disulfide bridges, and the molecular weight is estimated to be 6240 based on the amino acid composition. SPINK1, which binds rapidly to trypsin and inhibits its activity, is an important factor in the onset of pancreatitis [5].

1. SPINK1, WORKING AS A TRYPSIN INHIBITOR

Activation of Trypsinogen (Trypsin Production) and Inhibition of Trypsin Activity by SPINK1

Pancreatic digestive enzymes are stored as inactivated precursors (ie trypsin as trypsinogen) in pancreatic zymogen granules. Under normal conditions, activation is strictly controlled to prevent autodigestion of the pancreas (ie pancreatitis). However, in certain circumstances, excessive amounts of pancreatic trypsinogen are activated to trypsin (ectopic activation), activating other zymogens, and leading to autodigestion of the pancreas. Triggers for the activation of trypsinogen to trypsin in the pancreas include excessive pancreatic exocrine stimulation, reflux of bile or duodenal fluid, disturbance of pancreatic duct flow, and inflammation. Enterokinase is the most efficient activator, but other molecules that activate trypsinogen include trypsin (trypsin catalysed autoactivation), lysosomal enzyme cathepsin B and neutrophilic enzymes [5-8]. Also, activation of trypsin from trypsinogen can also occur without enzyme involvement (non-enzymatic autoactivation). Calcium inhibits the degradation (autolysis) of activated trypsin, whereas bile acids promote the autoactivation of trypsin. SPINK1 is synthesised in acinar cells of the pancreas and is thought to inhibit up to 20% of the trypsin activity in the pancreas by binding to its catalytic site. However, pancreatitis can ultimately develop, if pancreatic activation of trypsinogen is too high or the trypsin-binding ability of SPINK1 is too low [5].

Onset of Hereditary Chronic Pancreatitis by Cationic Trypsinogen and *SPINK1* Gene Mutations

Whitcomb *et al.* [6] determined the sequence of five exons of the cationic trypsinogen genes (protease serine 1; PRSS1) using genomic DNA from patients with hereditary chronic pancreatitis. They discovered a point mutation in exon 3 of the cationic trypsinogen gene (365GRA: R122H) that results in an amino acid substitution in the autolytic domain of trypsin. Thus, the mutation blocks autolysis and results in continuous trypsin activity.

*Address correspondence to this author at the Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan; Tel: +81-96-373-6596; Fax: +81-96-373-6599; E-mail: yamamura@gpo.kumamoto-u.ac.jp

1573-4080/09 $55.00+.00

Because balance of intrapancreatic balance between trypsin and SPINK1 activity is important for pancreatitis, it is hypothesised that mutations in the *SPINK1* gene that affect SPINK1 binding with trypsin will contribute to the onset of pancreatitis. There have been many reports of mutations in the *SPINK1* genes in patients with pancreatitis, and several hypothetical roles of these mutant proteins in pancreatitis [9-14]. A typical example is a substitution of Asn (AGT) with Ser (AAT) at position 34 in exon 3 (N34S). SPINK1 mutations are also found in normal people, and both homozygotes and heterozygotes are attributable to the development of pancreatitis. On the basis of these facts, some investigators recognize the role of the SPINK1 mutation as a disease modifier. However, the frequency of the N34S mutation occurring in patients with pancreatitis was considerably higher than that in people without pancreatitis [15]. Also, the rate of association of pancreatitis in people with the homozygotic N34S mutation was shown to be high (98%; 49/50) [15]. This high rate of association of pancreatitis in people with the homozygotic N34S mutation suggests that this mutation may be a recessive inherited trait.

Analysis of the Mechanism of Onset of Pancreatitis Using Genetically Altered Mice

To analyse the importance of trypsinogen activation (trypsin production) and its regulation by SPINK1 in the onset of pancreatitis, we generated the *Spink3* gene knockout mice by gene targeting and analysed their phenotypes [16]. The pancreatic acinar cells in knockout mouse showed excessive autophagy and disappeared completely after birth, indicating that Spink3 molecules are important in maintaining the integrity of pancreatic acinar cells. Interestingly, Chera *et al.* reported that similar phenotypes parallel in the endodermal epithelial cells observed upon silencing of the *Kazal1* gene in hydra [17]. In hydra, the endodermal epithelial cells carry out the digestive function together with the gland cells that produce zymogens and express the evolutionarily conserved gene *SPINK1*. A progressive *Kazal1* silencing induced excessive autophagy in the cytoplasm of digestive cells, and dramatic disorganization followed by a massive death of gland cells. These data suggests that the SPINK1 activity is required to prevent excessive autophagy in food digestive systems.

2. SPINK1, WORKING AS AUTOPHAGY REGULATOR

Autophagy; Bulk Degradation System in the Cell

Most intracellular short-lived proteins are selectively degraded by the ubiquitin-proteasome pathway [18, 19], while most long-lived proteins are degraded in lysosomes [20]. The general mechanism to deliver cytoplasmic components to the lysosomes is called autophagy (Fig. 1). The best understood role of autophagy is cellular housekeeping, a function that extends beyond the simple removal of damaged or unwanted products [21-23]. In fact, along with other proteolytic systems, lysosomes participate in the continuous turnover of intracellular constituents. Not only soluble cytosolic proteins but also organelles, such as mitochondria and peroxisomes, can be removed by autophagy [24-26]. In addition to maintaining cellular homeostasis, there is growing evidence for the participation of autophagy in processes such as cellular differentiation, tissue remodeling, growth control, cell defense and adaptation to adverse environments [27-30].

SPINK1 Regulates Autophagy in Acute Pancreatitis?

Vacuoles are found in pancreatic acinar cells of human acute pancreatitis and rodent experimental pancreatitis, but its origin has not been well understood. The vacuoles we observed by histological examination correspond to autophagosomes using autophagosome specific probe, microtubule-associated protein 1 light chain 3 (LC3-II) (Fig. 1), which are the hallmark of autophagy and contain zymogen granules in which trypsinogen exists physiologically [31]. What is the role of autophagy in acute pancreatitis? Two theoretical mechanisms of trypsinogen activation in pancreatic acinar cells have been proposed. In the "colocalization

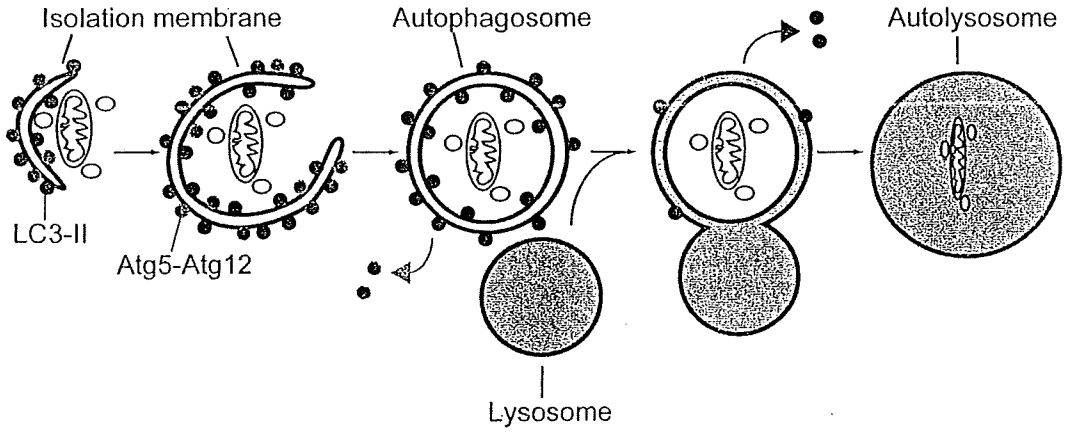

Fig. (1). Sheme of autophagy in mammalian cells. A portion of cytoplasm is enclosed by isolation membrane to form an autophagosome. Autophagosome fuses with lysosome to degrade the inside materials. The Atg12-Atg5 conjugate localized to the isolation membrane throughout its elongation process. LC3 is recruited to the membrane in the Atg5-dependent manner. Atg12-Atg5 dissociate from the membrane upon completion of autophagosome formation, while LC3 (-II) remains on the autophagosome membrane. Atg5 is required for elongation of the isolation membrane.

theory (cathepsin B theory)" [32-34], trypsinogen is activated by erroneous sorting of the lysosomal enzyme, cathepsin B. In the "autoactivation theory" [35], trypsinogen is transferred to an acidic environment in a subcellular compartment and activated by certain serine protease or independently of protease activity. We recently reported that conversion of trypsinogen to trypsin was greatly suppressed in autophagy-related gene 5 (*Atg5*; Fig. 1) deficient acinar cells, suggesting involvement of autophagy in trypsinogen activation [31]. We propose a third hypothetical mechanism of trypsinogen activation, autophagy theory [36]. Autophagy can provides both acidic and lysosomal hydrolase conditions. As SPINK1 recombinant protein both wild and mutated (N34S and R67C) have similar trypsin inhibition activity [37] (Fig. 2), it is possible that familial pancreatitis caused by mutation of the *SPINK1* gene is due to autophagy induction, but not to loss of binding to trypsin (Fig. 3).

3. SPINK1, WORKING AS A GROWTH FACTOR

Expression Pattern of *Spink3* During Mouse Embryonic Development

In human, SPINK1 is detected in many extrapancreatic tissues, including the stomach, colon, small intestine, liver, lung, kidney, and ovary [38-41]. To gain insight into its function, we analyzed the spatiotemporal expression profile of *Spink3*, using *in situ* hybridization and a *Spink3*$^{+/lacZ}$ knock-in mouse, in which *lacZ* was inserted into the *Spink3* locus [42]. *Spink3*^{lacZ} expression was first observed in the foregut, midgut, hindgut and the forebrain/midbrain junction region at 9.5 days post coitus (dpc). In the pancreas, *Spink3* mRNA was detected at 11.5 dpc, before formation of the typical shape of the exocrine structure of the pancreas. After differentiation of the intestinal tract, *Spink3*^{lacZ} expression was observed in the large intestine at 11.5 dpc, followed by expression in the small intestine at 13.5 dpc, before appearance of intestinal digestive enzymes. *Spink3* mRNA and *Spink3*^{lacZ} activity were also detected in other tissues, including the mesonephric tubules, urogenital ridge, genital swelling, and ductus epididymis. These data suggest that *Spink3* may play important roles in proliferation and/or differentiation of various cell types during development.

SPINK1 and Cancers

Tumor-associated trypsin inhibitor (TATI) was initially isolated from the urine of a patient with ovarian cancer [43]. TATI is a peptide produced at high concentrations by mucinous ovarian tumors, but it is expressed by several other tu-

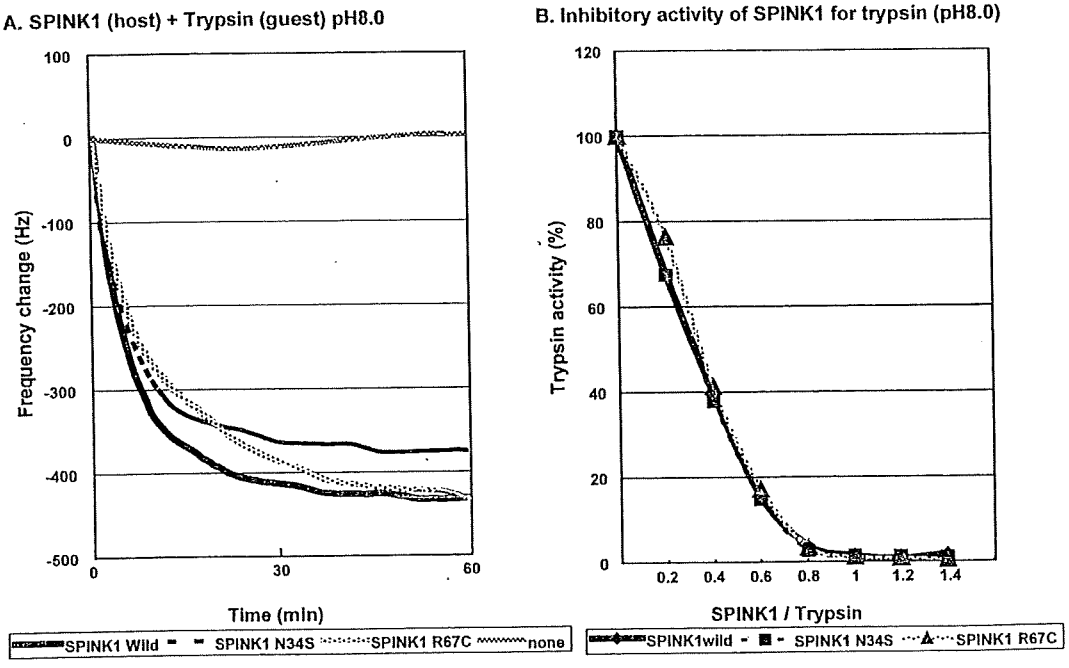

Fig. (2). (A) The binding affinity of trypsin with recombinant SPINK1 (wild, N34S, R67C) protein in comparison using a highly sensitive 27-MHz QCM (Initium, Tokyo, Japan) technique. In this method, qualitative and quantitative changes resulting from intermolecular interactions can be monitored and frequency (Hz) decreases in proportion to the change of molecular mass caused by binding of SPINK1, which are immobilized on the gold electrode surface, with trypsin. When trypsin was injected into the equilibrated solution containing SPINK1-immobilized sensor chip, the frequency decreased to about 400 Hz, suggesting that the binding affinity of SPINK1 N34S or R67C is almost same that of SPINK1 wild. (B) Trypsin inhibitory activity was determined from the residual trypsin activity after mixing bovine trypsin with recombinant SPINK1 (wild, N34S, R67C) protein (trypsin inhibitor [T.I.] / trypsin ratio ; 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4) in 0.1 M Tris-HCl buffer (pH 8.0) containing 0.02M $CaCl_2$ and 0.01% Triton X- 100 at 37°C, using *N*-Benzoyl-l-arginine *p*-nitroanilide (L-BAPA) [8]. After 10 min of incubation at 37°C, the reaction was stopped by adding 0.5 ml of 30% acetic acid, and the absorbance at 410nm was measured. Residual trypsin activity decreased with the increase in the molar ratio of SPINK1 wild to trypsin. SPINK1 N34S or R67C showed almost the same inhibitory activity for trypsin as did SPINK1 wild. At each trypsin inhibitor/trypsin ratio of 1, each residual trypsin activity had almost disappeared.

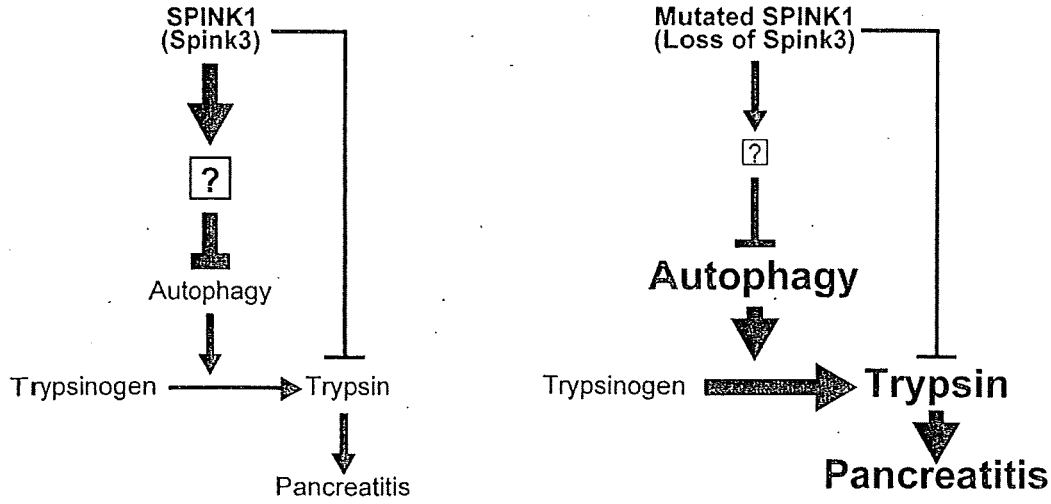

Fig. (3). Scheme of autophagy regulation by SPINK1 (Spink3). SPINK1 (Spink3) regulates both autophagy and activated trypsin in physiological condition. Mutated SPINK1 (loss of Spink3) can regulate activated trypsin, but not autophagy, which induces pancreatitis.

mors. TATI is identical to the SPINK1 [44]. Clinically, SPINK1 is most useful for monitoring of patients with mucinous ovarian cancer, but increased serum concentrations of SPINK1 may occur in many types of cancer [45]. In ovarian, bladder, and kidney cancer, SPINK1 is a marker of adverse prognosis. Tonouchi *et al.* reported that by the DNA microarray analysis, 13 differentially expressed genes were picked up, and quantitative RT-PCR reaction identified the SPINK1 as a candidate gene associated with early recurrence of intrahepatic cholangiocarcinoma after resection [46]. They described that the patients with higher levels of SPINK1 mRNA expression had significantly shorter recurrence-free survival. Recently, Tomlins *et al.* reported that an outlier-meta-analysis to identify SPINK1 outlier expression exclusively in a subset of ETS rearrangement-negative prostate cancers [47]. They demonstrated that SPINK1 outlier expression can be detected noninvasively in urine, and observed that SPINK1 outlier expression is an independent predictor of biochemical recurrence after resection.

SPINK1 Can Work as a Growth Factor *Via* Epidermal Growth Factor Receptor

Spink3 deficient pancreas shows no sign of regeneration of acinar cells [16], and SPINK1 silencing affects low budding rate in hydra [17], but its roles are not well known. There are some structural similarities between SPINK1 and the potent growth factor epidermal growth factor (EGF); both have similar numbers of amino-acid residues (56 and 53, respectively), molecular weights (about 6 kD), three intra-chain disulphide bridges (Fig. **4A**) and limited sequence homology [1]. There is 50% gene sequence homology between SPINK1 and EGF [48]. In 1985, Ogawa *et al.* [49] reported that SPINK1 was mitogenic for human fibroblasts. Some studies support the concept that SPINK1 binds to the EGF receptor (EGFR). Rat monitor peptide (rat homologue of SPINK1) has been reported to compete with mouse EGF for binding to the receptors of Swiss 3T3 fibroblasts [50] and an EGF receptor-blocking antibody removed the promigratory effects of SPINK1 on human HT-29 cells [51]. However, the structures of the SPINK1 and EGF are so different (Fig. **4B**), recently, we showed that SPINK1 binds to EGFR to activate its downstream signaling; resulting in proliferation of pancreatic and breast cancer cells (manuscript in submission). These data suggests that SPINK1 may act as growth factor *via* EGFR, as EGF families, in embryonic and cancer development.

4. SPINK1, WORKING AS A SPERM ACTIVITY REGULATION

Spink3 Binding Sites on Sperm Surface

Spink3 is strongly expressed in epitherial cells of seminal vesicle in male adult mice [42] (Fig. **5**). Chen *et al.* reported that Spink3 in mouse seminal vesicle, binds to the surface of sperm, and suppresses of Ca^{2+} uptake by spermatozoa [52]. It is well recognized that mammalian sperm from epididymis should undergo some Ca^{2+}-dependent modifications before fertilization. In the reproductive tract, the Ca^{2+} concentration is sufficient to elevate intracellular Ca^{2+} in the induction of these cell modifications at any time earlier than the sperm-egg encounter. However, earlier modifications before reaching the oviduct would cause spermatozoa to become infertile. Thus, the calcium movement across the membrane of spermatozoa should be prohibited at ejaculation until the cells reach the oviduct. Spink3-sperm binding leading to the suppression of Ca^{2+} movement across the cell membrane sheds some light into the function of the Spink3-binding sites. Future study is needed to elucidate the role of Spink3 as regulator of sperm activity.

CONCLUSIONS

Although, SPINK1 are expressed in variety tissues and solid tumors, the roles of each tissues and tumors are not known well. As Spink3 null deficient mice died within two weeks after birth, we cannot analyze the function of Spink3 in extra-pancreatic tissues at adulthood. In addition, whether SPINK1 mutations can cause hereditary chronic pancreatitis

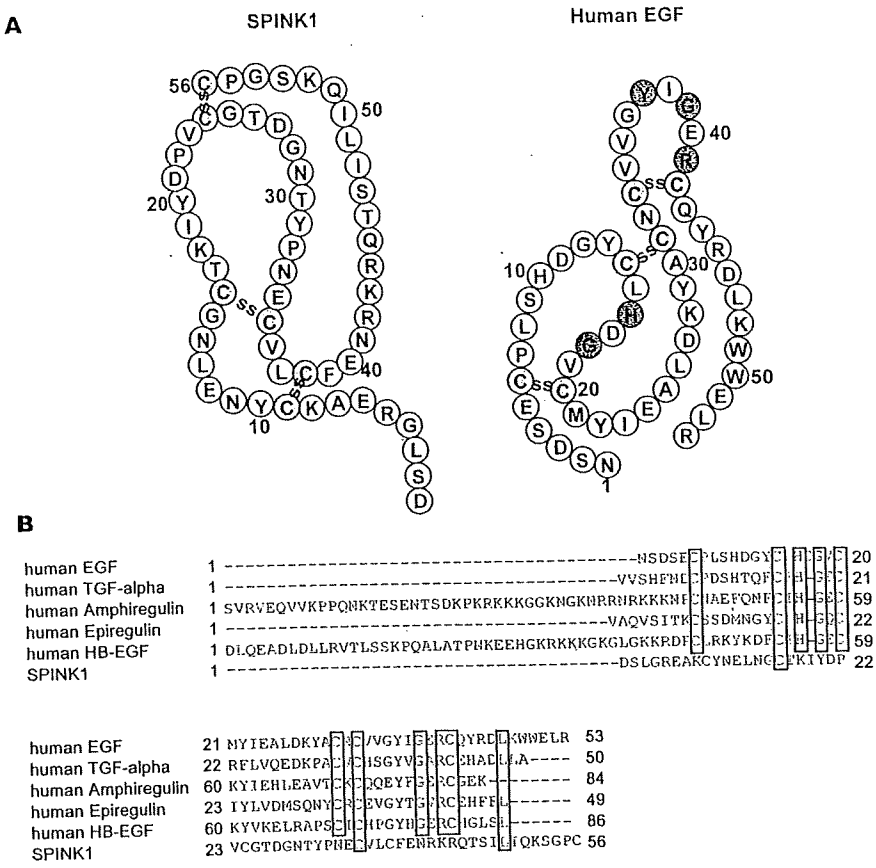

Fig. (4). (A) The comparison of SPINK1 and human EGF. Gray circles in human EGF indicate conserved in other EGF families. Cys residures (C) involved in the intrachain Cys-Cys disulphide bridge (SS). (B) The sequences of SPINK1 and EGF families. Amino acids identical in all structures are indicated by open boxes.

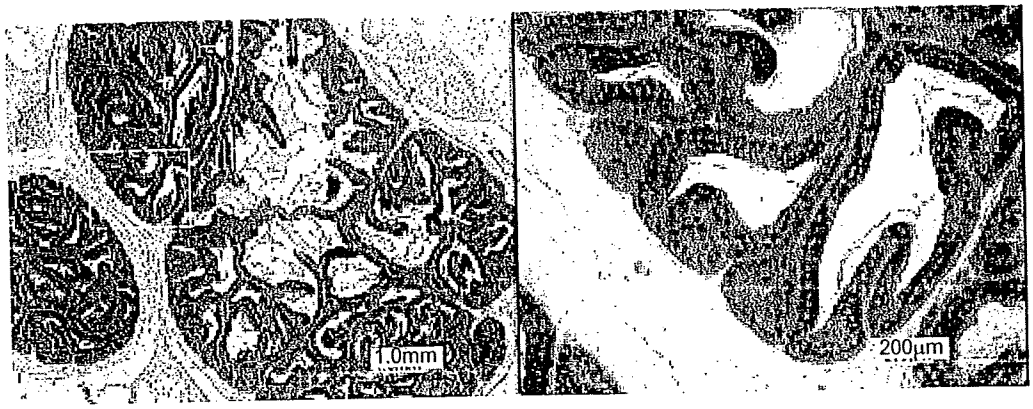

Fig. (5). Spink3 mRNA expression in mouse seminal vesicle. Spink3 strongly express epitherial cell in seminal vesicle (8 weeks old, C57BL/6J). Methods are previously described [42].

or not, is still unclear. In the next step, Spink3 conditional knockout models and human SPINK1 replacement models will be needed.

ACKNOWLEDGEMENTS

This work was supported in part by a KAKENHI (Grant-in-Aid for Scientific Research) in Priority Areas "Integrative Research Toward the Conquest of Cancer" and a Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a grant from the Osaka Foundation of Promotion of Clinical Immunology and a grant from Pancreas Research Foundation of Japan.

REFERENCES

[1] Marchbank, T.; Freeman, T.C.; Playford, R.J. Human pancreatic secretory trypsin inhibitor. Distribution, actions and possible role in mucosal integrity and repair. *Digestion*, 1998, *59*, 167-174.

[2] Kazal, L.A.; Spicer, D.S.; Brahinsky, R.A. Isolation of a crystalline trypsin inhibitor-anticoagulant protein from pancreas. *J. Am. Chem. Soc.*, **1948**, *70*, 304-340.

[3] Laskowski, M.; Wu, F.C. Temporary inhibition of trypsin. *J. Biol. Chem.*, **1953**, *204*, 797-805.

[4] Horii, A.; Kobayashi, T.; Tomita, N.; Yamamoto, T.; Fukushige, S.; Murotsu, T.; Ogawa, M.; Mori, T.; Matsubara, K. Primary structure of human pancreatic secretory trypsin inhibitor (PSTI) gene. *Biochem. Biophys. Res. Commun.*, **1987**, *149*, 635-641.

[5] Hirota, M.; Ohmuraya, M.; Baba, H. Genetic background of pancreatitis. *Postgrad. Med. J.*, **2006**, *82*, 775-778.

[6] Whitcomb, D.C.; Gorry, M.C.; Preston, R.A.; Furey, W.; Sossenheimer, M. J.; Ulrich, C. D.; Martin, S. P.; Gates, L. K., Jr.; Amann, S. T.; Toskes, P. P.; Liddle, R.; McGrath, K.; Uomo, G.; Post, J. C.; Ehrlich, G. D. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat. Genet.*, **1996**, *14*, 141-145.

[7] Greenbaum, L.M.; Hirshkowitz, A.; Shoichet, I. The activation of trypsinogen by cathepsin B. *J. Biol. Chem.*, **1959**, *234*, 2885-2890.

[8] Saluja, A.K.; Lerch, M.M.; Phillips, P.A.; Dudeja, V. Why does pancreatic overstimulation cause pancreatitis? *Annu. Rev. Physiol.*, **2007**, *69*, 249-269.

[9] Witt, H.; Luck, W.; Hennies, H.C.; Classen, M.; Kage, A.; Lass, U.; Landt, O.; Becker, M. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat. Genet.*, **2000**, *25*, 213-216.

[10] Kuwata, K.; Hirota, M.; Sugita, H.; Kai, M.; Hayashi, N.; Nakamura, M.; Matsuura, T.; Adachi, N.; Nishimori, I.; Ogawa, M. Genetic mutations in exons 3 and 4 of the pancreatic secretory trypsin inhibitor in patients with pancreatitis. *J. Gastroenterol.*, **2001**, *36*, 612-618.

[11] Pfutzer, R.H.; Barmada, M.M.; Brunskill, A.P.; Finch, R.; Hart, P.S.; Neoptolemos, J.; Furey, W.F.; Whitcomb, D.C. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology*, **2000**, *119*, 615-623.

[12] Chen, J.M.; Mercier, B.; Audrezet, M.P.; Raguenes, O.; Quere, I.; Ferec, C. Mutations of the pancreatic secretory trypsin inhibitor (PSTI) gene in idiopathic chronic pancreatitis. *Gastroenterology*, **2001**, *120*, 1061-1064.

[13] Kaneko, K.; Nagasaki, Y.; Furukawa, T.; Mizutamari, H.; Sato, A.; Masamune, A.; Shimosegawa, T.; Horii, A. Analysis of the human pancreatic secretory trypsin inhibitor (PSTI) gene mutations in Japanese patients with chronic pancreatitis. *J. Hum. Genet.*, **2001**, *46*, 293-297.

[14] Kuwata, K.; Hirota, M.; Nishimori, I.; Otsuki, M.; Ogawa, M. Mutational analysis of the pancreatic secretory trypsin inhibitor gene in familial and juvenile pancreatitis in Japan. *J. Gastroenterol.*, **2003**, *38*, 365-370.

[15] Hirota, M.; Kuwata, K.; Ohmuraya, M.; Ogawa, M. From acute to chronic pancreatitis: the role of mutations in the pancreatic secretory trypsin inhibitor gene. *JOP*, **2003**, *4*, 83-88.

[16] Ohmuraya, M.; Hirota, M.; Araki, M.; Mizushima, N.; Matsui, M.; Mizumoto, T.; Haruna, K.; Kume, S.; Takeya, M.; Ogawa, M.; Araki, K.; Yamamura, K. Autophagic cell death of pancreatic acinar cells in serine protease inhibitor Kazal type 3-deficient mice. *Gastroenterology*, **2005**, *129*, 696-705.

[17] Chera, S.; de Rosa, R.; Miljkovic-Licina, M.; Dobretz, K.; Ghila, L.; Kaloulis, K.; Galliot, B. Silencing of the hydra serine protease inhibitor Kazal1 gene mimics the human SPINK1 pancreatic phenotype. *J. Cell Sci.*, **2006**, *119*, 846-857.

[18] Hochstrasser, M. Ubiquitin-dependent protein degradation. *Annu. Rev. Genet.*, **1996**, *30*, 405-439.

[19] Hershko, A.; Ciechanover, A. The ubiquitin system. *Annu. Rev. Biochem.*, **1998**, *67*, 425-479.

[20] Mortimore, G.E.; Poso, A.R. Intracellular protein catabolism and its control during nutrient deprivation and supply. *Annu. Rev. Nutr.*, **1987**, *7*, 539-564.

[21] Seglen, P.O.; Bohley, P. Autophagy and other vacuolar protein degradation mechanisms. *Experientia*, **1992**, *48*, 158-172.

[22] Kim, J.; Klionsky, D.J. Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mammalian cells. *Annu. Rev. Biochem.*, **2000**, *69*, 303-342.

[23] Meijer, A. J. Amino acids as regulators and components of nonproteinogenic pathways. *J. Nutr.*, **2003**, *133*, 2057S-2062S.

[24] Lemasters, J.J.; Qian, T.; He, L.; Kim, J.S.; Elmore, S.P.; Cascio, W.E.; Brenner, D.A. Role of mitochondrial inner membrane permeabilization in necrotic cell death, apoptosis, and autophagy. *Antioxid Redox Signal.*, **2002**, *4*, 769-781.

[25] Bellu, A.R.; Kiel, J.A. Selective degradation of peroxisomes in yeasts. *Microsc. Res. Tech.*, **2003**, *61*, 161-170.

[26] Roberts, P.; Moshitch-Moshkovitz, S.; Kvam, E.; O'Toole, E.; Winey, M.; Goldfarb, D.S. Piecemeal microautophagy of nucleus in Saccharomyces cerevisiae. *Mol. Biol. Cell*, **2003**, *14*, 129-141.

[27] Hennig, K.M.; Neufeld, T.P. Inhibition of cellular growth and proliferation by dTOR overexpression in Drosophila: *Genesis*, **2002**, *34*, 107-110.

[28] Jacinto, E.; Hall, M.N. Tor signalling in bugs, brain and brawn. *Nat. Rev. Mol. Cell Biol.*, **2003**, *4*, 117-126.

[29] Melendez, A.; Talloczy, Z.; Seaman, M.; Eskelinen, E.L.; Hall, D.H.; Levine, B. Autophagy genes are essential for dauer development and life-span extension in C. elegans. *Science*, **2003**, *301*, 1387-1391.

[30] Otto, G.P.; Wu, M.Y.; Kazgan, N.; Anderson, O.R.; Kessin, R.H. Macroautophagy is required for multicellular development of the social amoeba Dictyostelium discoideum. *J. Biol. Chem.*, **2003**, *278*, 17636-17645.

[31] Hashimoto, D.; Ohmuraya, M.; Hirota, M.; Yamamoto, A.; Suyama, K.; Ida, S.; Okumura, Y.; Takahashi, E.; Kido, H.; Araki, K.; Baba, H.; Mizushima, N.; Yamamura, K. Involvement of autophagy in trypsinogen activation within the pancreatic acinar cells. *J. Cell Biol.*, **2008**, *181*, 1065-1072.

[32] Steer, M.L.; Meldolesi, J.; Figarella, C. Pancreatitis. The role of lysosomes. *Dig. Dis. Sci.*, **1984**, *29*, 934-938.

[33] Steer, M.L.; Meldolesi, J. The cell biology of experimental pancreatitis. *N. Engl. J. Med.*, **1987**, *316*, 144-150.

[34] van Acker, G.J.; Perides, G.; Steer, M.L. Co-localization hypothesis: a mechanism for the intrapancreatic activation of digestive enzymes during the early phases of acute pancreatitis. *World J. Gastroenterol.*, **2006**, *12*, 1985-1990.

[35] Leach, S.D.; Modlin, I.M.; Scheele, G.A.; Gorelick, F.S. Intracellular activation of digestive zymogens in rat pancreatic acini. Stimulation by high doses of cholecystokinin. *J. Clin. Invest.*, **1991**, *87*, 362-366.

[36] Ohmuraya, M.; Yamamura, K. Autophagy and acute pancreatitis: a novel autophagy theory for trypsinogen activation. *Autophagy*, **2008**, *4*, 1060-1062.

[37] Kuwata, K.; Hirota, M.; Shimizu, H.; Nakae, M.; Nishihara, S.; Takimoto, A.; Mitsushima, K.; Kikuchi, N.; Endo, K.; Inoue, M.; Ogawa, M. Functional analysis of recombinant pancreatic secretory trypsin inhibitor protein with amino-acid substitution. *J. Gastroenterol.*, **2002**, *37*, 928-934.

[38] Bohe, M.; Lindstrom, C.G.; Ohlsson, K. Varying occurrence of gastroduodenal immunoreactive pancreatic secretory trypsin inhibitor. *J. Clin. Pathol.*, **1987**, *40*, 1345-1348.

[39] Fukayama, M.; Hayashi, Y.; Koike, M.; Ogawa, M.; Kosaki, G. Immunohistochemical localization of pancreatic secretory trypsin inhibitor in fetal and adult pancreatic and extrapancreatic tissues. *J. Histochem. Cytochem.*, **1986**, *34*, 227-235.

[40] Matsuda, K.; Ogawa, M.; Murata, A.; Kitahara, T.; Kosaki, G. Elevation of serum immunoreactive pancreatic secretory trypsin inhibitor contents in various malignant diseases. *Res. Commun. Chem. Pathol. Pharmacol.*, **1983**, *40*, 301-305.

[41] Shibata, T.; Ogawa, M.; Takata, N.; Matsuda, K.; Niinobu, T.; Uda, K.; Wakasugi, C.; Mori, T. Distribution of pancreatic secretory trypsin inhibitor in various human tissues and its inactivation in the gastric mucosa. *Res. Commun. Chem. Pathol. Pharmacol.*, **1987**, *55*, 243-248.

[42] Wang, J.; Ohmuraya, M.; Hirota, M.; Baba, H.; Zhao, G.; Takeya, M.; Araki, K.; Yamamura, K. Expression pattern of serine protease inhibitor kazal type 3 (Spink3) during mouse embryonic development. *Histochem. Cell Biol.*, **2008**, *130*, 387-397.

[43] Huhtala, M.L.; Kahanpaa, K.; Seppala, M.; Halila, H.; Stenman, U.H. Excretion of a tumor-associated trypsin inhibitor (TATI) in urine of patients with gynecological malignancy. *Int. J. Cancer*, **1983**, *31*, 711-714.

[44] Huhtala, M.L.; Pesonen, K.; Kalkkinen, N.; Stenman, U.H. Purification and characterization of a tumor-associated trypsin inhibitor

from the urine of a patient with ovarian cancer. *J. Biol. Chem.*, 1982, *257*, 13713-13716.

[45] Stenman, U.H. Tumor-associated trypsin inhibitor. *Clin. Chem.*, 2002, *48*, 1206-1209.

[46] Tonouchi, A.; Ohtsuka, M.; Ito, H.; Kimura, F.; Shimizu, H.; Kato, M.; Nimura, Y.; Iwase, K.; Hiwasa, T.; Seki, N.; Takiguchi, M.; Miyazaki, M. Relationship between pancreatic secretory trypsin inhibitor and early recurrence of intrahepatic cholangiocarcinoma following surgical resection. *Am. J. Gastroenterol.*, 2006, *101*, 1601-1610.

[47] Tomlins, S.A.; Rhodes, D.R.; Yu, J.; Varambally, S.; Mehra, R.; Perner, S.; Demichelis, F.; Helgeson, B.E.; Laxman, B.; Morris, D.S.; Cao, Q.; Cao, X.; Andren, O.; Fall, K.; Johnson, L.; Wei, J.T.; Shah, R.B.; Al-Ahmadie, H.; Eastham, J.A.; Eggener, S.E.; Fine, S.W.; Hotakainen, K.; Stenman, U.H.; Tsodikov, A.; Gerald, W.L.; Lilja, H.; Reuter, V.E.; Kantoff, P.W.; Scardino, P.T.; Rubin, M.A.; Bjartell, A.S.; Chinnaiyan, A.M. The role of SPINK1 in ETS rearrangement-negative prostate cancers. *Cancer Cell*, 2008, *13*, 519-528.

[48] Yamamoto, T.; Nakamura, Y.; Nishide, J.; Emi, M.; Ogawa, M.; Mori, T.; Matsubara, K. Molecular cloning and nucleotide sequence of human pancreatic secretory trypsin inhibitor (PSTI) cDNA. *Biochem. Biophys. Res. Commun.*, 1985, *132*, 605-612.

[49] Ogawa, M.; Tsushima, T.; Ohba, Y.; Ogawa, N.; Tanaka, S.; Ishida, M.; Mori, T. Stimulation of DNA synthesis in human fibroblasts by human pancreatic secretory trypsin inhibitor. *Res. Commun. Chem. Pathol. Pharmacol.*, 1985, *50*, 155-158.

[50] Fukuoka, S.; Fushiki, T.; Kitagawa, Y.; Sugimoto, E.; Iwai, K. Competition of a growth stimulating-/cholecystokinin (CCK) releasing-peptide (monitor peptide) with epidermal growth factor for binding to 3T3 fibroblasts. *Biochem. Biophys. Res. Commun.*, 1987, *145*, 646-650.

[51] Marchbank, T.; Chinery, R.; Hanby, A.M.; Poulsom, R.; Elia, G.; Playford, R.J. Distribution and expression of pancreatic secretory trypsin inhibitor and its possible role in epithelial restitution. *Am. J. Pathol.*, 1996, *148*, 715-722.

[52] Chen, L.Y.; Lin, Y.H.; Lai, M.L.; Chen, Y.H. Developmental profile of a caltrin-like protease inhibitor, P12, in mouse seminal vesicle and characterization of its binding sites on sperm surface. *Biol. Reprod.*, 1998, *59*, 1498-1505.

Received: January 13, 2009 **Revised: February 11, 2009** **Accepted: February 13, 2009**

J Hepatobiliary Pancreat Surg (2009) 16:49–55
DOI 10.1007/s00534-008-0004-3

ORIGINAL ARTICLE

Prospective randomized controlled study of gastric emptying assessed by ^{13}C-acetate breath test after pylorus-preserving pancreaticoduodenectomy: comparison between antecolic and vertical retrocolic duodenojejunostomy

Kazuo Chijiiwa · Naoya Imamura · Jiro Ohuchida · Masahide Hiyoshi · Motoaki Nagano · Kazuhiro Otani · Masahiro Kai · Kazuhiro Kondo

Received: 16 November 2007 / Accepted: 16 January 2008 / Published online: 16 December 2008

Abstract
Background/Purpose To examine whether vertical retrocolic duodenojejunostomy is superior to antecolic duodenojejunostomy with respect to gastric emptying in a prospective, randomized, controlled study of patients undergoing pylorus-preserving pancreaticoduodenectomy (PpPD).
Methods Thirty-five patients undergoing PpPD between March 2005 and July 2007 were enrolled in the study. All provided informed consent. During PpPD, the patients were randomly assigned to either the antecolic (antecolic group, $n = 17$) or vertical retrocolic route (vertical retrocolic group, $n = 18$) just before the reconstruction. Each patient ingested ^{13}C-acetate in a liquid meal before surgery and on postoperative day (POD) 30. Gastric emptying variables (Tmax, T1/2) were determined and compared between groups.
Results Clinical delayed gastric emptying, defined as an inability of patients to take in an appropriate amount of solid food orally by POD 14, was found in 1 of 17 patients (6%) in the antecolic group and in 4 of 18 patients (22%) in the vertical retrocolic group, but the difference was not significant ($P = 0.34$). Tmax and T1/2 on POD 30 were prolonged in both groups in comparison to preoperative levels, but no significant difference was found between the two groups. Follow-up examinations revealed that gastric emptying had recovered to the preoperative level by POD 30 in approximately 80% of the patients, regardless of the reconstruction route.
Conclusions Vertical retrocolic duodenojejunostomy does not seem to offer an advantage with respect to gastric emptying.

Keywords Gastric emptying · Pylorus-preserving pancreaticoduodenectomy · Antecolic duodenojejunostomy · Vertical retrocolic duodenojejunostomy

K. Chijiiwa · N. Imamura · J. Ohuchida · M. Hiyoshi · M. Nagano · K. Otani · M. Kai · K. Kondo
Department of Surgical Oncology and Regulation of Organ Function, Miyazaki University School of Medicine, Kihara 5200, Kiyotake, Miyazaki, Japan

K. Chijiiwa (✉)
Department of Surgery, Miyazaki University School of Medicine, Kihara 5200, Kiyotake, Miyazaki, Japan
e-mail: kazuochi@med.miyazaki-u.ac.jp

Introduction

Pylorus-preserving pancreaticoduodenectomy (PpPD) is generally accepted as a standard operation for periampullary lesions. PpPD, in comparison to classic pancreaticoduodenectomy with hemigastrectomy, is reported to improve quality of life, nutritional status and weight gain without any difference in operative morbidity and mortality or in postoperative survival [1–4].

Delayed gastric emptying (DGE), however, is reported to be the most common and frustrating complication after PpPD. Despite the lack of a certain definition for DGE, the reported incidence varies from 20 to 60% [5–13]. DGE results in a prolonged hospital stay, which adds to hospital costs. Although DGE itself is not a fatal complication, minimizing DGE is important in patients undergoing PpPD.

Two reconstruction routes are used for duodenojejunostomy, the antecolic route and the retrocolic route. The reported incidence of DGE is >30% for the retrocolic route [12, 14, 15], whereas that for the antecolic route is <15%

[16, 17]. A recent prospective randomized controlled trial showed that DGE occurred in 50% of patients in whom the retrocolic route was used but in only 5% in whom the antecolic route was used [18]. These data suggest that the antecolic route is better. However, the 50% incidence of DGE associated with the retrocolic route seems high. We have shown that vertical retrocolic duodenojejunostomy, by which the stomach and duodenum are brought down through the left side of the transverse mesocolon in a straight, vertical manner, reduces the incidence of DGE [19].

Thus, a prospective randomized controlled trial was conducted to compare the incidence of clinical DGE and gastric emptying variables assessed by the ^{13}C-acetate breath test between patients who underwent antecolic duodenojejunostomy and those who underwent vertical retrocolic duodenojejunostomy. The aim of the study was to establish the superiority of the vertical retrocolic route with respect to gastric emptying after PpPD.

Patients and methods

Of 50 patients underwent pancreaticoduodenectomy at Miyazaki University Hospital between March 2005 and July 2007, 46 patients were scheduled to undergo PpPD. Patients were recruited into the study before surgery, on the basis of whether PpPD was anticipated and informed consent was obtained. Specific exclusion criteria included tumor infiltration into the duodenal bulb or presence of lymph node metastasis of the prepylorus ($n = 3$), failure to provide informed consent including the ^{13}C-acetate breath test ($n = 4$) were then excluded. Thus, 35 patients who underwent PpPD and consented to the protocol were enrolled in the study.

This prospective randomized controlled trial was approved by the ethical committee of our university hospital and informed consent was obtained from all patients. The randomization protocol involved assignment of patients to one of two reconstruction methods, the antecolic route and the vertical retrocolic route. Randomization took place during surgery before reconstruction. Gastric emptying was evaluated by means of the ^{13}C-acetate breath test just before surgery and on postoperative day (POD) 30.

Operative technique

The area resected during PpPD included the gallbladder, common hepatic duct, head of the pancreas, duodenum (except for the first portion), and 10 cm of the proximal jejunum. A few arcades of the right gastric artery and right gastroepiploic artery to the stomach were divided along the wall of the antrum (approximately 2–3 cm from the pyloric ring) for dissection of the peripyloric lymph nodes. The duodenum was freed from the surrounding tissue and transected approximately 4–5 cm distal to the pyloric ring. The lymph nodes in the hepatoduodenal ligament, the para-aortic lymph nodes, and those along the common hepatic artery and the right side of the superior mesenteric artery were dissected. The right gastric artery was divided at its origin in all patients. The left gastric artery and vein were carefully preserved. The lesser omentum close to the liver was dissected to allow free movement of the stomach. The vagal nerve, with the exception of the hepatic and pyloric branches, was preserved. These procedures allowed the stomach and the duodenum to be mobilized to the left in a straight, vertical manner.

As the first step in reconstruction, the proximal jejunum was brought through the right side of the transverse mesocolon by the retrocolic route. An end-to-side pancreaticojejunostomy was performed with duct-to-mucosal anastomosis. The pancreatic duct was anastomosed to the whole layer of the small opening in the jejunum to approximate the duct to the jejunal mucosa with the use of eight interrupted 5-0 PDS-II sutures (polydioxanone, Johnson & Johnson Co.), regardless of the size of the pancreatic duct. A 5-Fr polyethylene pancreatic drainage tube with a small knob (Sumitomo Bakelite Co., Japan) was placed in the pancreatic duct and exteriorized through the jejunal limb. The cut surface of the pancreas was then anastomosed to the jejunal seromuscular layer, and the end-to-side pancreaticojejunostomy was completed. A one-layer end-to-side hepaticojejunostomy with interrupted 5-0 PDS sutures was then performed 5–10 cm distal to pancreaticojejunostomy.

The final step was randomized to either to the antecolic route or vertical retrocolic route. For vertical retrocolic duodenojejunostomy, the left side of the transverse mesocolon (left side of the middle colic vessels) was opened, and the duodenum was brought down together with the gastric antrum in a straight, vertical manner. A retrocolic end-to-side duodenojejunostomy was performed at the caudal side of the transverse mesocolon and the antrum was fixed to the transverse mesocolon with a few 4-0 silk sutures. For antecolic duodenojejunostomy, the stomach was brought down antecolically. Braun anastomosis was added in both groups. Finally, the opening of the old ligament of Treitz and the jejunum brought up for pancreaticojejunostomy and hepaticojejunostomy were fixed to the mesocolon, and two or three closed drains were placed around the pancreatic and biliary anastomosis. All patients were given prophylactic antibiotics and H2 blocker postoperatively; none were given prokinetic drugs such as erythromycin.

Data collection and study endpoints

Clinicopathological data were collected prospectively for all patients. Data included postoperative mortality and morbidity, including pancreatic fistula, intraabdominal bleeding, pancreaticojejunostomy or hepaticojejunostomy leakage, intraabdominal abscess, and wound infection. Pancreatic fistula was defined when an amylase level in the fluid from the closed drains was >10,000 IU/l.

The first endpoint was clinical DGE defined as (1) the need for nasogastric tube decompression for more than 10 days (DGE 10), (2) the need for reinsertion of the nasogastric tube, or (3) an inability to take in an appropriate amount solid food orally by POD 14 (DGE 14), as described elsewhere [18].

The secondary endpoint was recovery of gastric emptying as assessed by ^{13}C-acetate breath test [20]. For at least 4 days before this test, all drugs, including H_2 blocker, were withdrawn. All patients ingested a liquid meal (200 Kcal/200 ml, RACOL, Ohtsuka Pharmaceutical Co., Tokyo, Japan) labeled with 100 mg sodium ^{13}C-acetate (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) in the morning after an overnight fast before surgery and on POD 30. Breath samples were collected in the collection bag (1.3 l) before and after ingestion of the test meal, i.e., before and at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, and 180 min after ingestion of the ^{13}C-acetate. The recovery of ^{13}C in the breath samples was analyzed by isotope-selective infrared spectrometry (UBiT IR 300, Otsuka Electronics Co., Ltd, Osaka, Japan). The time when $^{13}CO_2$ reached maximum excretion (Tmax) and half-emptying time (T1/2) were calculated by using analysis software (Microsoft Office Excel, Microsoft Japan, Tokyo, Japan).

Statistical analysis

All values are expressed as mean ± SD. Differences between groups were examined for statistical significance by chi-square test, unpaired or paired Student's t-test, Wilcoxon signed-rank test, or Mann–Whitney U test. Statistical analysis was performed by the statistician who was blind to the study group.

Results

Patient characteristics

Clinical characteristics of the enrolled patients are shown in Table 1. There were no statistical differences between the two groups in age, sex ratio, type of disease, percentage of patients with malignant disease, preoperative laboratory data including *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (BT-PABA) test value, percentage of patients with diabetes mellitus, HbA1c, operation time, or length of the remaining duodenum.

Table 1 Patient characteristics

	Duodenojejunostomy reconstruction route		
	Antecolic $n = 17$	Vertical retrocolic $n = 18$	*P* value
Age (years)	69.7 ± 11.0	66.9 ± 12.9	0.50
Male/female ratio	11/6	9/9	0.38
Hemoglobin (g/dl)	11.8 ± 1.3	12.3 ± 1.5	0.30
Serum albumin (g/dl)	3.67 ± 0.31	3.71 ± 0.46	0.76
Total cholesterol (mg/dl)	171.6 ± 37.4	179.4 ± 38.4	0.55
Diabetes mellitus (+/−)	5/12	2/16	0.23
BT-PABA test (%)	52.3 ± 18.2	49.2 ± 16.1	0.60
HbA1c (%)	5.7 ± 1.6	5.5 ± 0.9	0.67
Soft pancreas	9	10	0.88
Operation time (min)	602.6 ± 93.5	581.7 ± 76.5	0.48
Blood loss (ml)	1619.4 ± 914.9	1535.0 ± 877.7	0.78
Residual duodenum (cm)	3.7 ± 0.7	3.8 ± 0.5	0.54
Division of right gastric artery	17	18	0.54
Final diagnosis			
Benign/malignant disease	5/12	2/16	0.23
Bile duct cancer	8	6	
Pancreatic cancer	4	6	
Ampullary cancer	0	2	
Duodenal cancer	0	1	
IPMN	2	1	
Chronic pancreatitis	2	2	
Benign bile duct tumor	1	0	

Values are mean ± SD or number of patients

IPMN intraductal papillary mucinous neoplasm, *BT-PABA* *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid

Postoperative complications

As shown in Table 2, postoperative morbidity was observed in 9 of 17 patients (53%) in the antecolic group and 6 of 18 patients (33%) in the vertical retrocolic group. Intra-abdominal bleeding associated with pancreatic fistula and/or intra-abdominal abscess was observed in one patient in each group, and both patients were treated successfully by interventional transarterial embolization. Intra-abdominal abscess was the main complication and were treated successfully by drainage. No operative death or hospital death was observed.

Table 2 Postoperative outcomes

	Duodenojejunostomy reconstruction route		
	Antecolic $n = 17$	Vertical retrocolic $n = 18$	P value
Postoperative morbidity	9	6	0.24
Major P-J leakage	0	0	
Pancreatic fistula	1	1	0.97
H-J leakage	0	0	
Intra-abdominal bleeding	1	1	0.97
Intra-abdominal abscess	6	5	0.63
Wound infection	3	3	0.94
Respiratory dysfunction	0	0	
G-I bleeding	1	0	0.49
D-J leakage	0	0	
Mortality	0	0	
NG tube removed (POD)	1.2 ± 0.4	1.1 ± 0.3	0.59
DGE10	0	0	
Reinsertion of NG tube	0	0	
Liquid meal begun (POD)	5.4 ± 2.7	5.7 ± 2.4	0.72
Solid foods begun (POD)	8.4 ± 3.0	10.2 ± 5.1	0.21
DGE14	1	4	0.34
Postoperative stay (days)	40.8 ± 12.3	39.4 ± 11.1	0.74

P-J pancreaticojejunostomy, *H-J* hepaticojeunostomy, *G-I* gastrointestinal, *D-J* duodenojejunostomy, *NG* nasogastric

Clinical DGE

DGE clinically defined as DGE10 or DGE14 and the length of postoperative hospital stay are shown in Table 2. The nasogastric tube was removed on POD 1.2 ± 0.4 in the antecolic group on POD 1.1 ± 0.3 in the vertical retrocolic group. No patient needed a nasogastric tube for more than 10 days (DGE10), and reinsertion of a nasogastric tube was not necessary in any patient. The number of days to the start of liquid diet was similar between the two groups (5.4 days in the antecolic group and 5.7 days in the vertical retrocolic group). With respect to DGE14, one patient in the antecolic group and four in the vertical retrocolic group failed unlimited solid food oral intake by POD 14. Thus, the incidence of DGE defined as DGE14 was 6% (1 of 17 patients) in the antecolic group and 22% (4 of 18 patients) in the vertical retrocolic group. Although the rate was higher in the vertical retrocolic group, the difference did not reach statistical significance ($P = 0.34$). The overall incidence of DGE after PpPD was 14% (5 of 35 patients).

^{13}C-acetate gastric emptying test

Tmax did not differ between the vertical retrocolic group and the antecolic group before or on POD 30 ($P = 0.56$ before surgery and $P = 0.31$ on POD 30). Similarly, T1/2 did not differ between the two groups ($P = 0.99$ before surgery, $P = 0.26$ on POD 30) (Table 3). Neither reconstruction route had a significant effect on gastric emptying on POD 30 after PpPD.

Table 3 ^{13}C-Acetate gastric emptying test results

	Duodenojejunostomy reconstruction route		
	Antecolic $n = 17$	Vertical retrocolic $n = 18$	P value
Before surgery			
Tmax (h)	1.11 ± 0.25	1.08 ± 0.29	0.56
T1/2 (h)	1.78 ± 0.31	1.92 ± 0.81	0.99
After surgery (POD 30)			
Tmax (h)	1.54 ± 1.22	2.12 ± 2.14	0.31
T1/2 (h)	3.63 ± 3.15	6.21 ± 8.62	0.26

Tmax the time when $13CO_2$ reached maximum excretion, *T1/2* half emptying time

The ^{13}C-acetate gastric emptying test values before and on POD 30 were compared in each group. In the vertical retrocolic group, Tmax was significantly prolonged on POD 30 compared to that before surgery (2.12 ± 2.14 h versus 1.08 ± 0.29 h, $P < 0.02$), whereas no significant difference was found in the antecolic group (1.54 ± 1.22 h versus 1.11 ± 0.25 h, $P = 0.29$). However, T1/2 was significantly longer in each group on POD 30 compared to the corresponding preoperative value ($P = 0.0023$ in the antecolic group, $P = 0.0002$ in the vertical retrocolic group (Table 3). Gastric emptying was not completely restored to the preoperative level in either group by POD 30. Mean Tmax on POD 30 in the antecolic group was increased 1.39-fold, and that in the vertical retrocolic group was increased 1.96-fold. Similarly, T1/2 was increased 2.04-fold in the antecolic group and 3.23-fold in the vertical retrocolic group. Greater increases in Tmax and T1/2 were observed in the vertical retrocolic group than in the antecolic group.

Tmax before and after surgery in each patient is shown in Fig. 1. Individual changes in Tmax before and after surgery were similar to individual changes in T1/2. A greater than twofold increase in Tmax was observed in 3 (18%) of 17 patients in the antecolic group, and in 4 (22%) of 18 patients in the vertical retrocolic group. Tmax of all patients before surgery ($n = 35$) was 1.09 ± 0.26 h, ranging from 0.7 to 1.5 h. Tmax greater than 1.5 h on POD 30 was found in four patients in each group. Tmax on POD 30 remained similar to the preoperative level in most patients (approximately 80%) in both groups.

Discussion

The present study showed that the incidence of clinical DGE was lower with the antecolic route than with the vertical

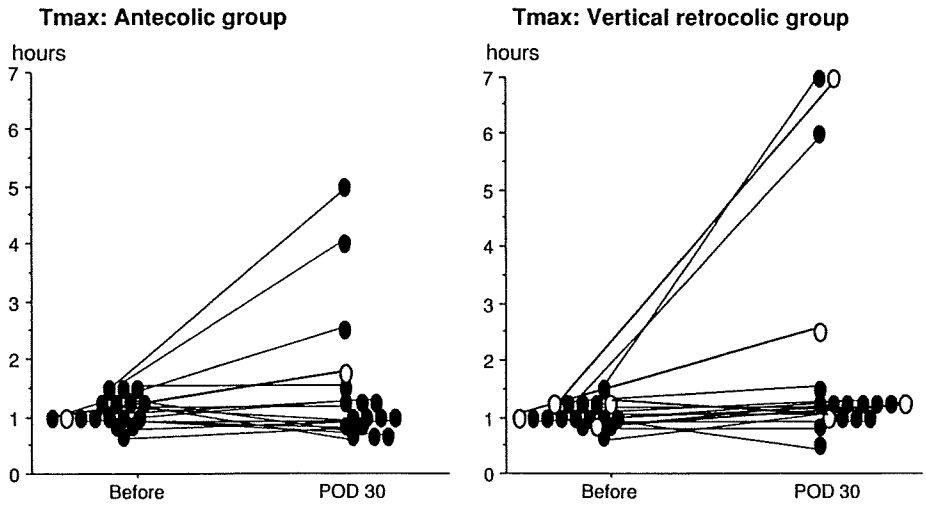

Fig. 1 Changes in Tmax of individual patients (before surgery versus POD 30). *Open circles* represent patients who were not able to tolerate appropriate solid food by POD 14 (DGE14)

retrocolic route, but the difference was not significant (6% with the antecolic route versus 22% with the vertical retrocolic route, $P = 0.34$). Moreover, gastric emptying (Tmax, T1/2) as assessed by the ^{13}C-acetate breath test did not differ significantly between the antecolic route and the vertical retrocolic route before or on day 30 after PpPD. T1/2 was significantly prolonged in both groups after PpPD, indicating that gastric emptying remained impaired on POD 30, regardless of the reconstruction route. The degree of impairment was greater in patients in whom vertical retrocolic reconstruction was performed. An analysis of individual patients revealed that on POD 30, gastric emptying was similar to the preoperative level in approximately 80% of patients, regardless of the reconstruction route.

Since Traverso and Longmire [1] first reported PpPD in 1978, the procedure has been accepted as a standard procedure for periampullary diseases. This is because it yields better quality of life, nutritional status, and weight gain without any difference in postoperative survival than the Whipple procedure [1–4, 21]. The postoperative mortality rate has fallen recently, but complications associated with pancreaticoduodenectomy remain, the most troublesome of which are pancreatic fistula, intra-abdominal infection, intra-abdominal bleeding, wound infection, and DGE. DGE was first reported by Warshaw and Torchiana [5]. Postoperative DGE decreases patient comfort, increases the risk of aspiration pneumonia, prolongs hospital stay, and increases medical costs.

DGE is considered a specific complication of PpPD, because it is specifically attributed to pylorus-sparing resection of the pancreatic head [5–7, 10]. Several underlying mechanisms have been proposed: (1) gastric atony or gastroparesis caused by vagotomy, resection of the duodenal pacemaker, or disruption of the gastroduodenal neural connections [11], (2) local ischemic injury of the antrum and pylorus [7], (3) gastric atony in response to a reduced circulating levels of motilin [12], (4) torsion or angulation of the reconstructed alimentary tract [7], (5) gastric dysrhythmia or gastroparesis secondary to an intraabdominal complication such as anastomotic leakage, abscess, or local inflammation [15, 21]. Recent studies have shown that DGE does not occur as a result of pylorus preservation but rather as a consequence of postoperative complications [17, 22, 23]. Although the exact mechanism underlying DGE is not clear, our results suggest that DGE is related to clinical or even subclinical local inflammation caused by postoperative complications; three of our five patients with DGE (DGE14) had abscess or pancreatic fistula.

DGE has been generally defined as DGE10 (need for a nasogastric tube for more than 10 days) and DGE14 (failure to tolerate solid food by POD 14). The reported incidence of DGE ranges from 20 to 60% [5–13]. In the present study, no patient needed nasogastric decompression for more than 3 days. The nasogastric tube was removed on POD 1 in 30 (86%) of the 35 patients and on POD 2 in the remaining five. None required reinsertion of a nasogastric tube. With respect to DGE14, failure to tolerate solid food was observed in 5 of 35 patients, for an overall incidence of 14%.

A difference in DGE with respect to the reconstruction route, whether antecolic or retrocolic duodenojejunostomy, has been reported. In a retrospective study, Park et al. [23]. found that the incidence of DGE was 31.7% in the retrocolic group, but only 6.5% in the antecolic group. Hartel et al. [24]. reported an incidence of 24% with the retrocolic route and 5% with the antecolic route. Sugiyama et al. [25]. reported that DGE occurred in 1 of 12 patients (8%) in the antecolic group, but in 13 of 18 patients (72%) in the retrocolic group. These retrospective studies have suggested that the incidence of DGE is lower with the antecolic route than with the retrocolic route. A recent prospective randomized study by Tani et al. [18]. yielded an incidence of 50% for the retrocolic route, but 5% for the antecolic route. In the current prospective randomized

controlled trial, the incidence of DGE was 22% with the vertical retrocolic route and 6% with the antecolic route, but the difference was not statistically significant. Although the purpose of this study was to show the superiority of the vertical retrocolic route, an interim analysis did not show any advantage of the vertical retrocolic route; hence, we decided to terminate the study.

In addition to clinically defined DGE, the ^{13}C-acetate gastric emptying test showed that gastric emptying on POD 30 did not differ between the antecolic route and the vertical retrocolic route. Moreover, the gastric emptying did not recover to the preoperative level by 30 days in approximately 20% of patients, regardless of the reconstruction route. A greater increase in Tmax and T1/2 was observed with the vertical retrocolic route than with the antecolic route. These results suggest that the vertical retrocolic route offers no advantage. An analysis of the individuals showed that gastric emptying variables (Tmax, T1/2) had recovered to the preoperative level in approximately 80% of patients on POD 30, regardless of the reconstruction route. The day of analysis and type of meal selected (POD 30, liquid meal) should be reconsidered in another study.

In conclusion, the incidence of DGE and gastric emptying variables (Tmax, T1/2) after PpPD were similar between patients in whom reconstruction was performed by the antecolic route and those in whom it was performed by the vertical retrocolic route. On POD 30, gastric emptying was impaired in both groups compared to the preoperative level, but an analysis of individuals showed that it had recovered to the preoperative level in most patients, regardless of the reconstruction route.

Acknowledgments A part of this study was supported by grant-in-aid (No. 17591417) from Japanese Ministry of Education, Culture, Sports, Science and Technology. Dr. Imamura is an equally contributed first author.

References

1. Traverso LW, Longmire WP Jr. Preservation of the pylorus in pancreaticoduodenectomy. Surg Gynecol Obstet. 1978;146:959–62.
2. Kozuschek W, Reith HB, Waleczek H, Haarmann W, Edelmann M, Sonntag D. A comparison of long term results of the standard Whipple procedure and the pylorus preserving pancreatoduodenectomy. J Am Coll Surg. 1994;178:443–53.
3. Zerbi A, Balzano G, Patuzzo R, Calori G, Braga M, Carlo VD. Comparison between pylorus-preserving and Whipple pancreatoduodenectomy. Br J Surg. 1995;82:975–9.
4. Mosca F, Giulianotti PC, Balestracci T, Candio GD, Pietrabissa A, Sbarana F. Long-term survival in pancreatic cancer: pylorus-preserving versus Whipple pancreatoduodenectomy. Surgery. 1997;122:553–66.
5. Warshaw AL, Torchiana DL. Delayed gastric emptying after pylorus-preserving pancreaticoduodenectomy. Surg Gynecol Obstet. 1985;160:1–4.
6. Jiminez RE, Fernandez-del Castillo C, Rattner DW, Chang YC, Warshaw AL. Outcome of pancreaticoduodenectomy with pylorus preservation or with antrectomy in the treatment of chronic pancreatitis. Ann Surg. 2000;231:293–300.
7. Itani KM, Coleman RE, Meyers WC, Akwari OE. Pylorus-preserving pancreaticoduodenectomy. A clinical and physiological appraisal. Ann Surg. 1986;204:655–64.
8. Yeo CJ, Cameron JL, Sohn TA, Lillemoe KD, Pitt HA, Talamini MA. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s: pathology, complications, and outcomes. Ann Surg. 1997;226:248–57.
9. Fabre JM, Burgel JS, Navarro F, Boccarat G, Lemoine C, Domergue J. Delayed gastric emptying after pancreaticoduodenectomy and pancreaticogastrostomy. Eur J Surg. 1999;165:560–65.
10. Patel AG, Toyama MT, Kusske AM. Pylorus-preserving resection for pancreatic cancer. Is it any better? Arch Surg. 1995;130:838–43.
11. Braasch JW, Deziel DJ, Rossi RL, Watkins E Jr, Winter PF. Pyloric and gastric preserving pancreatic resection: experience with 87 patients. Ann Surg. 1986;204:411–8.
12. Yeo CJ, Barry K, Sauter PK, Sostre S, Lillemoe KD, Pitt HA, et al. Erythromycin accelerates gastric emptying after pancreaticoduodenectomy. Ann Surg. 1993;218:229–37.
13. Yamaguchi K, Tanaka M, Chijiiwa K, Nagakawa T, Imamura M, Takada T. Early and late complications of pylorus-preserving pancreatoduodenectomy in Japan 1998. J Hepatobiliary-Pancreat Surg. 1999;6(3):303–11.
14. Lin P, Lin YJ. Prospective randomized comparison between pylorus-preserving and standard pancreaticoduodenectomy. Br J Surg. 1999;86:603–7.
15. van Berge Henegouwen MI, van Gulik TM, de Wit LT, Allema JH, Rauws EA, Obertop H, et al. Delayed gastric emptying after standard pancreaticoduodenectomy versus pylorus-preserving pancreaticoduodenectomy: an analysis of 200 consecutive patients. J Am Coll Surg. 1997;185:373–9.
16. Neoptolemos JP, Russell RC, Bramhall S, Theis B. Low mortality following resection for pancreatic and periampullary tumours in 1026 patients: UK surgery of specialist pancreatic units. Br J Surg. 1997;84:1370–6.
17. Horstmann O, Markus PM, Ghadimi MB, Becker H. Pylorus preservation has no impact on delayed gastric emptying after pancreatic head resection. Pancreas. 2004;28:69–74.
18. Tani M, Terasawa H, Kawai M, Ina S, Hirono S, Uchiyama K, et al. Improvement of delayed gastric emptying in pylorus-preserving pancreaticoduodenectomy Results of a prospective, randomized, controlled trial. Ann Surg. 2006;243:316–20.
19. Chijiiwa K, Ohuchida J, Hiyoshi M, Nagano M, Kai M, Kondo K. Vertical retrocolic duodenojejunostomy decreases delayed gastric emptying after pylorus-preserving pancreatoduodenectomy. Hepato Gastroenterol. 2007;54:1874–7.
20. Braden B, Adams S, Duan LP, Orth KH, Maul FD, Lembcke B, et al. The [13C] acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. Gastroenterology. 1995;108:1048–55.
21. Hocking MP, Harrison WD, Sninsky CA. Gastric dysrhythmias following pylorus-preserving pancreaticoduodenectomy. Possible mechanism for early delayed gastric emptying. Dig Dis Sci. 1990;35:1226–30.
22. Riediger H, Makowiec F, Schareck WD, Hopt UT, Adam U. Delayed gastric emptying after pylorus-preserving pancreatoduodenectomy is strongly related to other postoperative complications. J Gastrointest Surg. 2003;7:758–65.
23. Park YC, Kim SW, Jang JY, Ahn YJ, Park YH. Factors influencing delayed gastric emptying after pylorus-preserving pancreatoduodenectomy. J Am Coll Surg. 2003;196:859–65.

24. Hartel M, Wente MN, Hinz U, Kleef J, Wagner M, Muller MW, et al. Effect of antecolic reconstruction on delayed gastric emptying after the pylorus-preserving Whipple procedure. Arch Surg. 2005;140:1094–9.

25. Sugiyama M, Abe N, Ueki H, Masaki T, Mori T, Atomi Y. A new reconstruction method for preventing delayed gastric emptying after pylorus-preserving pancreatoduodenectomy. Am J Surg. 2004;187:743–6.