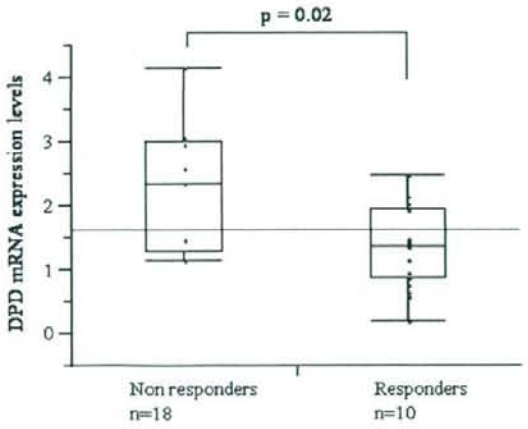

Fig. 2 DPD mRNA expression levels between responders and non-responders. Responders are defined as the serum CA19-9 level fell by at least 50% (18 patients), and non-responders are defined as their serum CA19-9 either decreased by less than 50% or increased (10 patients). DPD expression levels in responders were significantly lower than those in non-responders

phase II clinical trials of patients with colorectal cancer. This rate was not appreciably higher than the response rate with conventional 5-fluorouracil-based combination therapy. In pancreatic cancer, however, we previously obtained a response rate of higher than 50% with a combination of S-1 and cisplatin, which was considerably better than the response rate with conventional 5-fluorouracil treatment. Takechi et al. demonstrated that the in vitro antitumor activity of 5-FU against tumor cells with low DPD expression levels is not appreciably affected by the addition of CDHP. Against tumor cells with high DPD expression, however, the 50% inhibitory concentration differed by about twofold [25]. In tumors with low DPD expression such as colorectal cancer, the presence of CDHP provides no benefit, and the response to S-1 is similar to that with 5-fluorouracil. In tumors with high DPD expression such as pancreatic cancer, S-1 may be more effective than 5-fluorouracil.

Studies of DPD expression in patients with pancreatic cancer are scarce. Mori et al. used enzyme-linked immunosorbent assay [17] to measure DPD protein expression in various types of tumors and reported that pancreatic cancer had high DPD expression levels, similar to cancer of the neck, liver, esophagus, and breast [17]. Kamoshida et al. [14] used immunostaining to measure DPD levels in various types of tumors. They found that DPD expression was high in pancreatic cancer and low in colorectal cancer. To our knowledge, expression of DPD mRNA has not been assessed previously in pancreatic cancer. Uetake et al. reported that DPD gene expression correlated with DPD enzyme activity in colorectal cancer [27]. Mori et al. [17] showed that DPD enzyme activity strongly correlated with DPD protein expression as measured by ELISA. They found that the level of DPD protein expression was about threefold higher in pancreatic cancer and twofold higher in gastric cancer than in colorectal cancer, consistent with the mRNA levels measured by RT-PCR. These results suggest that the mRNA, protein, and activity levels of the DPD gene strongly correlate. DPD mRNA levels can be measured even in small specimens obtained by endoscopy or needle biopsy. The concomitant use of laser-captured microdissection allows DPD mRNA expression of tumors to be assessed quantitatively and objectively, providing clinically significant advantages over conventional procedures.

In our study, DPD levels were significantly lower in the responders to S-1 as assessed by the change in tumor marker levels than in the non-responders (1.25 vs. 2.20; $P = 0.02$). The median DPD level in the non-responders was 2.20, equivalent to about twofold that in the responders and fourfold that in colorectal cancer. Ichikawa et al. measured DPD mRNA levels in patients with gastric cancer and reported that the anticancer activity of S-1 does not depend on the level of DPD expression because CDHP inhibits DPD [13]. Although, the median DPD level did not differ significantly between responders and non-responders to S-1 in their study, patients in whom DPD expression exceeded a certain level did not respond to S-1. The inhibitory activity of CDHP against DPD is about 130-fold higher than that of uracil. However, in patients whose tumors have very high levels of DPD, the inhibitory effect of CDHP on DPD may be limited. In patients with such tumors, more potent DPD inhibitors should be used, or drugs with other mechanisms of action, such as gemcitabine, should be used instead of fluoropyrimidines. As for the former, eniluracil, a new, more potent DPD inhibitor, was developed and evaluated in clinical trials. Eniluracil decreases DPD enzyme activity by more than 99% in the liver and reduces 5-fluorouracil clearance by more than 18-fold [2]. In a phase II study of eniluracil combined with 5-fluorouracil in patients with pancreatic cancer, the median survival was only 3.6 months, indicating an unsatisfactory outcome. Moreover, 68% of the patients had grade 3 or higher toxicity. Subsequent clinical development was therefore terminated Rothenberg, 2002. Because eniluracil itself is free of serious toxicity, the toxic effects in clinical trials were attributed to prolonged, high serum concentrations of 5-fluorouracil. These results suggested the limitations of combination therapy with 5-fluorouracil and DPD inhibitors.

Our data may provide a molecular biologic basis for the high antitumor activity of S-1 in patients with pancreatic cancer. Gemcitabine has been shown to be somewhat effective against pancreatic cancer [3], but its anticancer activity

is far from satisfactory. Further studies of S-1, in patients with pancreatic cancer are awaited.

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Preoperative endoscopic pancreatic stenting: a novel prophylactic measure against pancreatic fistula after distal pancreatectomy

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Abstract
Background/Purpose. The prevention of pancreatic fistula is still a major problem in distal pancreatectomy (DP). We have recently adopted preoperative endoscopic pancreatic stenting with the aim of preventing the leakage of pancreatic juice from the resection plane of the remnant pancreas after DP. We reviewed ten patients who underwent this intervention.
Methods. One to 6 days before surgery, the patients underwent an endoscopic transpapillary pancreatic stent (7 Fr., 3 cm) placement. The perioperative short-term outcomes were assessed.
Results. Preoperative endoscopic pancreatic stenting was successfully performed in all ten patients. Two (20%) patients, both with intraductal papillary mucinous tumor, developed mild acute pancreatitis after the stent placement. None of the ten patients developed pancreatic fistula. The pancreatic stent was removed 8–28 days (mean, 11 days) postoperatively.
Conclusions. Preoperative endoscopic pancreatic stenting may be an effective prophylactic measure against pancreatic fistula development following DP.

Key words Pancreatic fistula · Distal pancreatectomy · Endoscopic pancreatic stenting

Introduction

Pancreatic fistula is a common complication of distal pancreatectomy (DP).[1] The incidence of pancreatic fistula after DP ranges from 5% to 60%.[2–15] Recent advances in medical and surgical care in DP cannot completely eliminate the possibility of pancreatic fistula development. Most pancreatic fistulas usually resolve spontaneously, albeit over a lengthy period, after conservative treatment including pancreatic rest (no oral intake with total parenteral nutrition or octreotide administration) and adequate drainage (drains placed during the initial operation or postoperatively via the percutaneous approach).[16–18] However, some patients with pancreatic fistula develop complications, such as intraabdominal abscess, sepsis, and lethal hemorrhage. For pancreatic fistulas refractory to conservative treatment, surgical intervention is indicated, but this is often technically difficult and associated with a high morbidity rate (13%).[19] Therefore, prophylactic strategies can be employed to decrease the incidence of pancreatic fistula after DP.[20]

We previously reported the case of a patient in whom preoperative endoscopic pancreatic stenting prevented pancreatic fistula development following local pancreatic resection,[21] and another case of a patient in whom endoscopic pancreatic stenting was effective for a pancreatic pseudocyst that developed after DP.[22] Taken together, these experiences suggested that preoperative endoscopic pancreatic stenting might be envisaged as a prophylactic measure against pancreatic fistula after DP. Thus, we have recently adopted this intervention with the aim of preventing the leakage of pancreatic juice from the resection plane of the remnant pancreas after DP.[23] This preoperative endoscopic intervention is simple and easy and appears to minimize the incidence of pancreatic fistula after DP.[23] In this article, we present the short-term outcomes of ten patients who underwent preoperative endoscopic pancreatic stenting for the prophylaxis of pancreatic fistula after DP.

Patients and methods

Ten patients underwent preoperative endoscopic pancreatic stenting for the prophylaxis of pancreatic fistula after DP. The patients were five men and five women with a mean age of 58 years (range, 27–80 years). One to 6 days (mean, 5 days) before surgery, the patients

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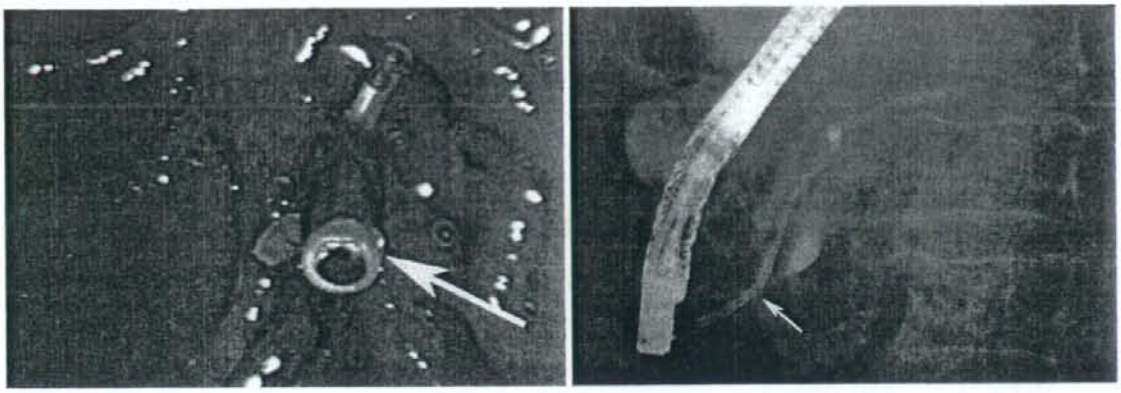

Fig. 1. A Duodenoscopic view of the papilla of Vater after pancreatic stent (*arrow*) placement. **B** Retrograde pancreatogram after endoscopic pancreatic stent (*arrow*) placement

underwent an endoscopic transpapillary pancreatic stent placement (Geenen, 7 Fr., 3 cm; Wilson-Cook Medical, Winston-Salem, NC, USA) (Fig. 1A,B). Before applying pancreatic stenting, informed consent was obtained from each patient.

The indications for DP included ductal adenocarcinoma ($n = 4$), intraductal papillary mucinous tumor ($n = 3$), ductal adenocarcinoma concomitant with intraductal papillary-mucinous tumor ($n = 1$), and mucinous cystic tumor ($n = 2$) of the pancreas. A concomitant splenectomy was carried out in all ten patients. During the pancreatic transection, the pancreatic parenchyma was divided using an electrocautery and/or a surgical scalpel (electrocautery, 4; scalpel, 2; both, 2), and the main pancreatic duct was ligated with a nonabsorbable suture. Pancreatic ducts ranged from 2 to 4 cm (mean, 3 cm) in diameter. The stump of the pancreas was not closed and was left open. Fibrin glue was not applied to the stump. One or two closed suction drains were placed in the splenic fossa adjacent to the pancreatic stump. Octreotide was not administered postoperatively.

The definition of pancreatic fistula was a drain output of any measurable volume of fluid on or after postoperative day 3 with an amylase content greater than three times the serum amylase level.[24] Other potential major complications after DP were categorized and defined as follows: intraabdominal infection (drainage of purulent but amylase-poor fluid); intraabdominal abscess (need for percutaneous or surgical drainage)[25]; and late postpancreatectomy hemorrhage (intraabdominal hemorrhage 24 h or more after operation).[26]

Results

Preoperative endoscopic pancreatic stenting was successfully performed in all ten patients. Endoscopic sphincterotomy or balloon dilatation was not required in any of the patients. The proximal tip of the stent was placed in the main pancreatic duct of the pancreatic head. Two patients, both with intraductal papillary mucinous tumor, developed mild acute pancreatitis the day after the stent placement. Both patients underwent endoscopic pancreatic stenting 6 days before the surgery. These patients were treated conservatively by pancreatic rest and protease inhibitor administration.

Postoperatively, none of ten patients developed pancreatic fistula. Other major complications including intraabdominal infection or abscess, and late postpancreatectomy hemorrhage did not occur in these ten patients. The pancreatic stent was removed 8–28 days postoperatively (mean, 10 days). No pancreatic stent occlusion was found by macroscopic observation of the stent lumen.

Discussion

The standard surgical technique for preventing the leakage of pancreatic juice from the resection plane of the remnant pancreas after DP is reportedly the ligation of the main pancreatic duct and closure by suturing of the resection plane of the remnant pancreas.[27] However, the resection plane has several stumps of the branch pancreatic ducts communicating with the main duct.[4] Konishi et al.[4] reported a mean number of two such branches, ranging from zero to five branches. These small ducts cannot all be identified and ligated during transection because they have small diameters. Furthermore, the closure of the resection plane of the remnant pancreas by suturing may not occlude such small ducts. These may lead to postoperative pancreatic fistula.[21] The prevention of pancreatic fistula is therefore still a major problem in DP.

Endoscopic pancreatic stenting has been used to treat pancreatic ductal stricture, pancreatolithiasis, pancreas divism, and pancreatic duct disruption (fistula and pseudocyst development secondary to acute or chronic pancreatitis, or pancreatic trauma).[28–30] However, only a few reports have described endoscopic pancreatic stenting for the treatment or prophylaxis of pancreatic fistula development after a pancreatic surgery.[21–23,29,31] In this article, we have presented the short-term outcomes of preoperative endoscopic pancreatic stenting as a prophylactic measure against pancreatic fistula after DP. The rationale for this new approach was based on previous results showing that (1) endoscopic treatment (pancreatic sphincterotomy and/or pancreatic stenting) is effective for treating pancreatic fistula[29,31] or pseudocyst[22] development after DP; and (2) preoperative endoscopic pancreatic stenting prevented pancreatic fistula after local pancreatic resection.[21] The results reported herein show that endoscopic pancreatic stenting is a minimally invasive technique that may prevent pancreatic fistula after DP, because 100% of patients who underwent this procedure actually did not develop pancreatic fistula. A pancreatic stent seems to allow the site of pancreatic leakage to seal via a pancreatic decompression effect by abolishing the pressure gradient between the pancreatic duct and the duodenum.[21,22,28,29] However, it could not be confirmed whether the pancreatic stent itself prevented pancreatic fistula. Further investigation of more cases is required to determine the value of this approach, and the randomization of patients to undergo either DP with versus without pancreatic stenting will be warranted to determine the actual efficacy of this approach.

The early complications associated with the stent placement are noteworthy. Two of four (50%) patients with intraductal papillary mucinous tumor developed acute mild pancreatitis after stent placement. This type of tumor secretes mucin; therefore, copious mucin fills the main and/or branch pancreatic ducts. Mucin can be associated with the stagnation of the pancreatic juice in a pancreatic stent. This stagnation of the pancreatic juice may result in the development of acute pancreatitis. To this end, preoperative stenting seems unsuitable for patients with this type of tumor, but intraoperative stenting (by intraoperative duodenoscopy or via duodenostomy) following removal of the tumor can be applicable to them.[21] We have previously experienced the case of a patient in whom intraoperative pancreatic stenting via duodenostomy prevented pancreatic fistula development after local pancreatic resection.[21]

Although no pancreatic stent occlusion was found in this series, a stent occlusion may directly induce postoperative pancreatic fistula development. If technically possible, large and short (7 Fr., 3 cm) stent placement, as in the present cases, seems desirable. Long-term stent placement may induce pancreatic ductal changes that resemble chronic pancreatitis.[32] Pancreatic fistula usually occurs 2–7 days after surgery.[2,16] Therefore, a stent should be retrieved within 2 weeks of surgery unless a pancreatic fistula has developed.[21]

In conclusion, preoperative endoscopic pancreatic stenting may be an effective prophylactic measure against pancreatic fistula after DP in selected patients.

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ORIGINAL ARTICLE

Falciform ligament in pancreatoduodenectomy for protection of skeletonized and divided vessels

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Abstract
Background/purpose Pancreatic fistula, which is one of the main causes of late postpancreatectomy hemorrhage (PPH), is a common complication of pancreatoduodenectomy (PD). It may erode the anastomosis site and vascular wall in its vicinity, resulting in pseudoaneurysm formation and/or the rupture of major vessels. To protect the vessels near the area for pancreaticojejunostomy from potential pancreatic fistula, we have adopted a surgical option by which such vessels are separated from the pancreaticojejunostomy using a pedicled falciform ligament. We reviewed 36 patients who underwent PD that included this option.
Methods After the PD was completed (before reconstructions), the pedicled falciform ligament was spread widely on the major vessels exposed during resection, and was fixed to the surrounding retroperitoneal connective tissue. These procedures enabled the complete separation of these vessels from the pancreaticojejunostomy.
Results The mobilization and placement of the falciform ligament in the space between the pancreaticojejunostomy and the major vessels were successfully carried out without any complications. Although ten (28%) patients developed pancreatic fistula and three (8%) developed intraabdominal infection, none of the patients developed late PPH.
Conclusions The present surgical option is technically simple and easy, and may be an effective prophylactic measure against late PPH following PD.

Keywords Pancreatic fistula · Pancreatoduodenectomy · Falciform ligament · Late postpancreatectomy hemorrhage

Introduction

Pancreatic fistula is a common complication of pancreatoduodenectomy (PD) [1]. It exposes skeletonized or divided vessels directly to pancreatic juice and/or intraabdominal infection, creating a condition for vessel erosion and late postpancreatectomy hemorrhage (late PPH) [2, 3]. The protection of these vessels from pancreatic fistula and/or intraabdominal infection is therefore an important surgical option for preventing late PPH. The placement of an omental flap between skeletonized or divided vessels and the pancreaticojejunostomy may be a useful surgical technique for protecting these vessels [3–6]. However, an appropriate omental flap may not always be obtained. We previously reported that the wrapping of the vessels near the pancreatic stump, using a pedicled falciform ligament, was an effective prophylactic measure against late PPH following distal pancreatectomy [7]. Based on this experience, we have recently adopted a surgical option in PD by which skeletonized or divided vessels are separated from the pancreaticojejunostomy using a pedicled falciform ligament. This surgical option is simple and easy, and appears to prevent late PPH following PD. We herein present our procedure, which involves the use of the falciform ligament, and we review the short-term operative outcomes of 36 patients who underwent PD that included this option.

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Methods and patients

Surgical techniques for use of the falciform ligament

A pedicled falciform ligament was easily and rapidly obtained during a midline abdominal incision [7]. After the incising of the linea alba, the preperitoneal fat is bluntly dissected to the right prior to incising the peritoneum. The falciform ligament is mobilized by dividing it near the umbilicus and incising its anterior peritoneal reflections along the posterior right rectus sheath. An additional length is obtained by continuing the anterior incision cephalad to the undersurface of the diaphragm to the triangular ligament and incising the posterior peritoneal reflections cephalad to the anterior surface of the liver to the triangular ligaments [8]. Note that the pedicled falciform ligament (Fig. 1a) normally reaches the space between the pancreaticojejunostomy and the major vessels exposed during resection. After completion of the PD (before reconstructions), the pedicled falciform ligament is spread widely on the major vessels, such as the common/proper hepatic artery, superior mesenteric vein (SMV), portal vein (PV), splenic vein, and stump of the gastroduodenal artery (Fig. 1b), and fixed with interrupted 3-0 silk sutures to the surrounding retroperitoneal connective tissues (Fig. 1c). This procedure enables the complete separation of these vessels from the pancreaticojejunostomy.

Patients and pancreatoduodenectomy

Between October 2002 and February 2007, 36 patients without prior history of upper abdominal surgery underwent PD employing the placement of a pedicled falciform ligament. The patients were 23 men and 13 women with a mean age of 65 years (range 22–78 years). The indications for surgery included pancreatic ductal cancer (n = 11), intraductal papillary-mucinous tumor of the pancreas (n = 2: adenoma, 1; carcinoma, 1), intraductal papillary-mucinous carcinoma of the pancreas concomitant with intrahepatic bile duct cancer (n = 1), serous cystadenoma of the pancreas (n = 1), solid and pseudopapillary tumor of the pancreas (n = 1), cancer of the papilla of Vater (n = 7), duodenal cancer (n = 2), and bile duct cancer (n = 11). Of the 36 patients, 23 (64%) had normal (soft) pancreas.

Pancreatoduodenectomy included two types: standard PD (n = 17) and pylorus-preserving PD (n = 19). For standard PD, antrectomy was performed. Two patients underwent concomitant resections of other organs: lateral segmentectomy of the liver in one (a patient with intraductal papillary-mucinous carcinoma of the pancreas concomitant with intrahepatic bile duct cancer) and transverse colectomy in one (a patient who showed severe inflammatory adhesion between intraductal papillary-mucinous carcinoma of the pancreas and the transverse mesocolon). PV resection and reconstruction were carried out in four patients with pancreatic ductal cancer. Lymph

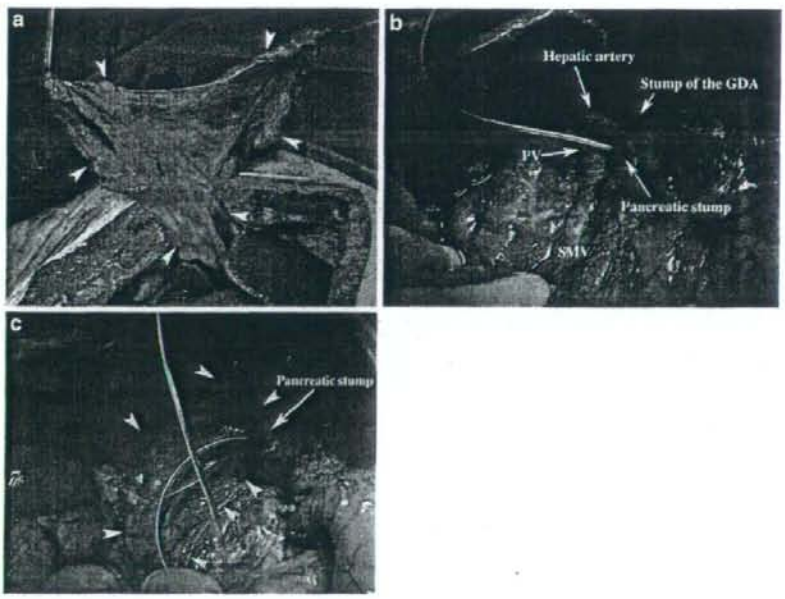

Fig. 1 Protection of skeletonized and divided vessels using falciform ligament. a The pedicled falciform ligament (*arrowheads*). b Surgical view before the placement of the pedicled falciform ligament. The hepatic artery, stump of the gastroduodenal artery (*GDA*), superior mesenteric vein (*SMV*), and portal vein (*PV*) are exposed. C Surgical view after the placement of the pedicled falciform ligament (*arrowheads*). This procedure enables the complete separation of the vessels from the pancreaticojejunostomy

nodes in the hepatoduodenal ligament and around the common hepatic artery were dissected in the patients with pancreatic ductal cancer, bile duct cancer, duodenal cancer, and cancer of the papilla of Vater. The SMV to the PV was skeletonized in all the patients. In the patients with pancreatic ductal cancer, the nerve plexus around the superior mesenteric artery (SMA) was dissected over lengths of approximately 5 cm, up to the origin of the SMA. None of the patients underwent paraaortic lymph node dissection.

The pancreas was transected at the level of the left side of the SMV. During the pancreatic transection, the pancreatic parenchyma was divided using a Harmonic Scalpel (Ethicon Endo-Surgery, Cincinnati, OH, USA) or a surgical scalpel (Harmonic Scalpel, $n = 10$; surgical scalpel, $n = 16$).

Reconstruction was carried out according to a modification of the method described by Child. Pancreaticojejunostomy was performed, using end-to-side anastomosis, in two different ways depending on the diameter of the main pancreatic duct. When the diameter was large (4 mm and more), the pancreatic stump was anastomosed to the jejunum in two layers: the duct-to-mucosa and pancreatic parenchyma-to-jejunal seromuscular layer ($n = 17$). When the diameter was small (3 mm or less), the main pancreatic duct was intubated using a retrograde transjejunal pancreatic tube, with pursestring sutures around the duct and the stab wound in the jejunum, and then the pancreatic parenchyma was anastomosed to the jejunal seromuscular layer ($n = 19$). The common hepatic duct was anastomosed end-to-side to the jejunum 6–8 cm distal to the pancreaticojejunostomy. The duodenum or the stomach was then anastomosed end-to-side to the jejunum. Finally, a Braun jejunostomy was performed near the duodeno- (gastro-) jejunostomy. After completion of the reconstruction, closed suction drains were placed near the biliary and pancreatic anastomoses. The tip of the drain near the pancreatic anastomosis was placed in the compartment between the pancreatic anastomosis and the falciform ligament that was spread widely on the major vessels. No octreotide was administered postoperatively.

Definition of postoperative complications

The definition of pancreatic fistula was a drain output of any measurable volume of fluid on or after postoperative day 3 with an amylase content greater than three times the serum amylase level [9]. Other complications were categorized and defined as follows: intraabdominal infection (drainage of purulent but amylase-poor fluid); intraabdominal abscess (need for percutaneous or surgical drainage) [6]; delayed gastric emptying (inability to return to a standard diet by the end of the first postoperative week or the need for nasogastric intubation for 10 days or more) [10, 11]; bile leakage (bilious drainage) [12]; intestinal obstruction (need for the insertion of a long tube); and late PPH (intraabdominal hemorrhage 24 h or more after the operation) [13].

Results

The mobilization and placement of the falciform ligament in the space between the pancreaticojejunostomy and the major vessels were successfully carried out without any complications. In none of patients did the pedicled falciform ligament show ischemia at the end of the operation. The mean operating time and blood loss were 445 min (range 258–887 min) and 687 ml (range 110–1,554 ml), respectively. Postoperative complications occurred in 15 of the 36 patients in the series, for an overall postoperative morbidity rate of 42%. There were no postoperative deaths.

Ten patients (28%) developed pancreatic fistula. According to the grading system of pancreatic fistula proposed by the International Pancreatic Fistula Study Group, three were defined as grade B pancreatic fistula and seven as grade A. All ten patients with pancreatic fistula had a soft pancreas. None of the patients with pancreatic fistula required surgical or percutaneous interventions. The pancreatic fistula resolved spontaneously after conservative treatments including pancreatic rest and/or intermittent local irrigation with saline via the drain. Although three patients (8%) developed intraabdominal infection, none of the patients required additional drainage. The intraabdominal infections resolved after conservative treatments including antibiotic administration and local irrigation with saline via the drain. Delayed gastric emptying was observed in two patients (6%), and there was a small intestinal obstruction in one patient (3%). A reoperation (adhesionolysis) was carried out in one patient with intestinal obstruction. Other major complications, including bile leakage, wound abscess, and late PPH did not occur.

Discussion

Late PPH still remains a fatal complication after PD [2]. The incidence of PPH after PD ranges from 1 to 8% [2, 6, 13–17], and the mortality rates associated with PPH are high, ranging from 11 to 58% [13–21]. Late PPH is reportedly most often caused by pancreatic fistula and/or intraabdominal infection [2, 6, 22–24]. These may erode the anastomosis site and vascular wall in its vicinity, resulting in pseudoaneurysm formation and/or the rupture of major vessels such as hepatic arteries, the stump of the gastroduodenal artery, and portal vessels [2]. Of note, pancreatic fistula, which is one of the main causes of late

PPH, is a common complication of PD [1, 9]. Its reported incidence varies from 2 to 28% [9, 12]. Recent advances in medical and surgical care in PD cannot completely eliminate the possibility of pancreatic fistula development [1]. The prevention of late PPH associated with pancreatic fistula is therefore an important step toward improving the short-term outcome after PD.

The falciform ligament is the obliterated umbilical vein (ligamentum teres or round ligament) and its encompassing parietal peritoneum [8]. The pedicled falciform ligament adequately mobilized is therefore a large (15–30 cm) autologous tissue that will usually reach any surgical area in the upper abdomen. It is therefore sufficiently large to cover the major vessels widely. Although its suitability is not widely appreciated, it has been used in several types of abdominal operations, including those for hepatic injury [8] or perforated gastroduodenal ulcer [25], and hiatal herniorrhaphy [26]. Only a few reports have been published on the use of the falciform ligament in pancreatic surgery [7, 27]. We previously showed that the wrapping of the vessels near the pancreatic stump, using a pedicled falciform ligament, was successful for reducing the incidence of late PPH (0%) after distal pancreatectomy [7]. Baker et al. [27]. described the use of the falciform ligament for the protection of the vessels in PD. However, the outcomes of the use of the falciform ligament in PD have not yet been described.

Others [3–6] use an omental flap, whose suitability is widely appreciated, for the same purpose as ours. Indeed, the placement of an omental flap between the major vessels and the pancreaticojejunostomy is successful for reducing the incidence of late PPH (0–1%) after PD [3–6]. However, an adequate omentum is not available in some patients [8]. Because the space between the pancreaticojejunostomy and the major vessels is small, a thick omentum with rich adipose tissue seems to be inappropriate. Complications associated with the use of an omental flap, such as intestinal obstruction, total flap necrosis, and infection, have been reported [6]. Therefore, we consider that the falciform ligament is an excellent alternative to the omentum for the protection of the major vessels.

The presented surgical option, which is the placement of a pedicled falciform ligament between the pancreaticojejunostomy and the major vessels, is a simple and easy technique for the complete separation of the vessels from the pancreaticojejunostomy. It is suggested that the falciform ligament can prevent the diffusion of pancreatic juice with or without bacterial infection and protect the vessels. In the present study, although 28% of the patients developed pancreatic fistula, none of the patients developed late PPH. However, it could not be confirmed whether the present surgical option itself prevented late PPH. Further controlled randomized studies involving large numbers of patients are required to confirm the value of this surgical option.

In conclusion, the present surgical option (the placement of a pedicled falciform ligament between the pancreaticojejunostomy and major vessels) is technically simple and easy, and we consider that it may prevent late PPH caused by pancreatic fistula following PD.

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SURGEON AT WORK

Modified liver hanging maneuver with extraparenchymal isolation of the middle hepatic vein in left hepatectomy

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Abstract
Background/purpose The liver hanging maneuver has been employed mostly for right hepatectomy. After the space between the inferior vena cava and the liver parenchyma is bluntly dissected, a tape is passed through the space, with the upper end of the tape between the right hepatic vein and middle hepatic vein (MHV). We devised a modified maneuver with tape repositioning between the MHV and left hepatic vein (LHV) after extraparenchymal isolation of the MHV, in left hepatectomy with the caudate lobe and without the MHV.
Methods The ligamentum venosum is divided. Traction of the cranial stump of the ligament in the cranial and left direction shows an avascular plane between the LHV and MHV, and enables the taping of each MHV and LHV. Finally, the upper end of the hanging tape is repositioned.
Results This maneuver was successfully performed in three patients.
Conclusions The present maneuver is feasible and useful in left hepatectomy.

Keywords Liver hanging maneuver · Ligamentum venosum · Hepatic vein

Introduction

The liver hanging maneuver proposed by Belghiti et al. [1] has been employed mostly for right hepatectomy [2–7]. In the original method, the space between the anterior surface of the inferior vena cava (IVC) and the liver parenchyma is bluntly dissected without liver mobilization. Then a tape is passed through the space, with the upper end of the tape between the right hepatic vein (RHV) and middle hepatic vein (MHV). This tape serves as a guide to the transection plane and as a means of suspending the liver, facilitating the control of bleeding in the deeper parenchyma of the liver and protecting the IVC [1–7].

Only a few articles have described modified hanging maneuvers for left hepatectomy with or without the MHV [7, 8]. For left hepatectomy without the MHV, it is rational to pass a hanging tape just on the left side of the MHV, contrary to the original hanging maneuver. Kim et al. [6] reported a method in which a tape is passed along the anterior surface of the retrohepatic IVC between the MHV and left hepatic vein (LHV) after dividing the liver parenchyma between the MHV and LHV using an ultrasonic dissector. We devised a modified liver hanging maneuver with tape repositioning between the MHV and LHV after extraparenchymal isolation of the MHV, in left hepatectomy with the caudate lobe and without the MHV. This technique obviates the need for liver parenchymal dissection before passing the hanging tape.

Method

After laparotomy, intraoperative ultrasonography is performed, with special attention to confirm the absence of tumor infiltration to the IVC, abnormal short hepatic veins in the space to be dissected, or abnormal branches near the roots of the LHV and MHV [4, 9]. The anterior surface of the suprahepatic IVC is exposed, and the space between the RHV and MHV is dissected 2–3 cm in the caudal direction,

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using a right-angled dissector. The caudal edge of the caudal lobe is lifted from the IVC, and small short hepatic veins are divided up to the level of the inferior right hepatic vein. A long light curved Kelly clamp is inserted behind the caudate lobe, just to the left side of the right inferior hepatic vein, and passed cranially along the anterior surface of the IVC between the 10 and 11 o'clock position, under ultrasonographic guidance [4]. The Kelly clamp is advanced further toward the tip of the operator's left index finger inserted into the previously dissected space between the RHV and MHV. Then, a tape for liver hanging is passed (Fig. 1).

The left triangular and coronary ligaments and the lesser omentum are divided. The anterior surface of the junction of the LHV and MHV is dissected. After the left lobe is lifted up, the ligamentum venosum (Arantius' ligament) is ligated and divided at the cranial one-third. The cranial stump of the ligament is grasped with the Kelly clamp and dissected cranially toward the IVC, almost until the ligament broadens into its attachment (the posterior wall of the LHV or the common trunk of the LHV and MHV). Traction of the ligament in the cranial and left direction puts the walls of the LHV and MHV under tension, showing an avascular plane between the posterior walls of the LHV and MHV and the liver parenchyma. This plane is developed toward the anterior aspect of the dissection. Another Kelly clamp is inserted between the LHV and MHV in the posteroanterior direction, and the LHV is encircled with a tape (Figs. 2, 3). Furthermore, a tunnel is created behind the common trunk of the LHV and MHV. Consequently, extraparenchymal taping of the MHV is easily performed. Finally, the upper end of the hanging tape previously passed between the liver and IVC is repositioned from between the RHV and MHV to between the MHV and LHV, using the tape around the MHV (Fig. 4).

After cholecystectomy, the hepatic hilum is dissected to free the right and left Glisson's pedicles, in the inferior surface of the quadrate lobe. Then, the lower end of the hanging tape is passed between both pedicles. This

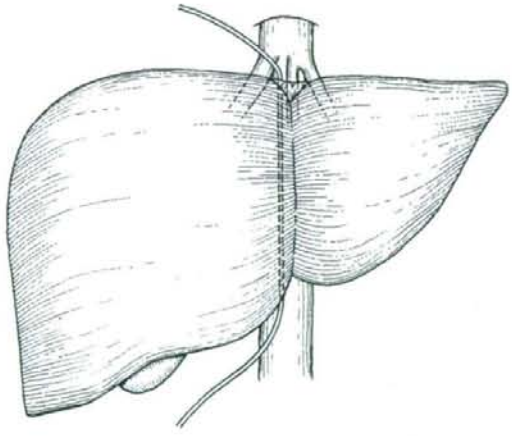

Fig. 1 The hanging tape is passed along the anterior surface of the retrohepatic inferior vena cava (IVC) between the right hepatic vein (RHV) and middle hepatic vein (MHV)

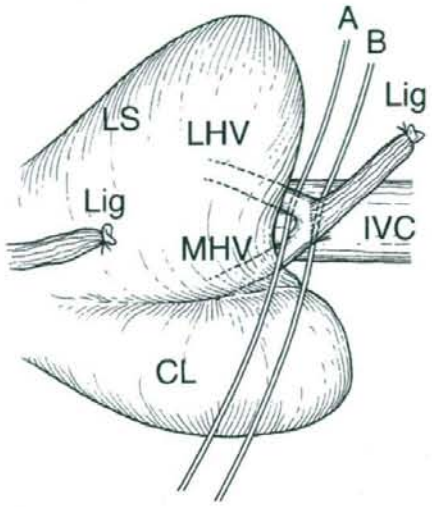

Fig. 2 The ligamentum venosum (*Lig*) is ligated and divided. Traction of the cranial stump of the ligament in the cranial and left direction reveals an avascular plane between the posterior walls of the left hepatic vein (*LHV*) and *MHV* and the liver parenchyma. The LHV is encircled with a tape (*A*). The common trunk of the LHV and MHV is also encircled (*B*). *LS* left lateral segment, *CL* caudate lobe, *IVC* inferior vena cava

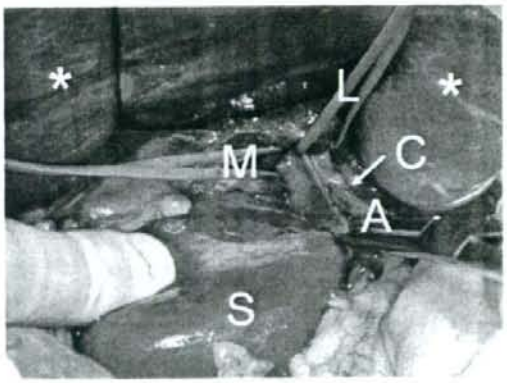

Fig. 3 Intraoperative view of the liver from the *left side*, while the left lobe (*asterisk*) is lifted up. Each MHV (*M*) and LHV (*L*) is encircled. *A* ligamentum venosum (Arantius' ligament), *C* common trunk of the LHV and MHV, *S* spiegel lobe of the caudate lobe

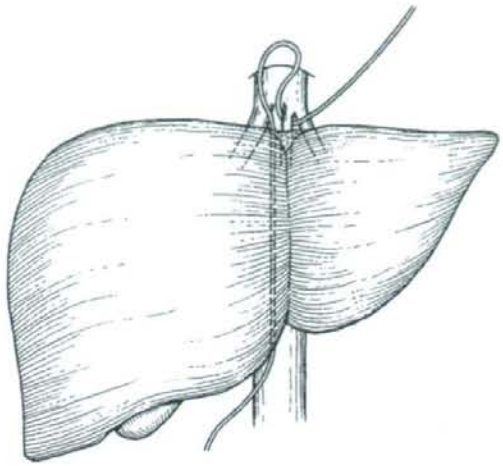

Fig. 4 The upper end of the hanging tape is repositioned between the MHV and LHV, using the tape around the MHV

repositioning of the tape is more effective than previously used positions for suspension of the caudate lobe. Hepatic parenchymal transection is then performed with Pringle's maneuver.

Results

Three patients underwent left hepatectomy with the caudate lobe and without the MHV, by using the present modified liver hanging maneuver. There were two men and one woman, with a mean age of 66 years (range 58–74 years). The diagnoses were: hilar cholangiocarcinoma ($n = 2$) and colorectal liver metastasis ($n = 1$). The maneuver was successfully performed. The mean operative time was 260 min (range 176–315 min). The mean intraoperative blood loss was 642 ml (range 320–811 ml). None of the patients required blood transfusion during or after the operation.

Discussion

The present technique is a combination of the original liver hanging maneuver and the extraparenchymal isolation of the MHV. The latter is an application of the maneuver of Majno et al. [9] namely, extraparenchymal isolation of the LHV by dividing and pulling the ligamentum venosum.

Previously, it had been believed that the extraparenchymal isolation of each LHV and MHV was technically difficult. However, Majno et al. [9] proposed the maneuver of extraparenchymal isolation of the LHV. This maneuver is based on the anatomical knowledge that the fibers of the ligamentum venosum insert not on the IVC itself but on the posterior wall of the LHV or the common trunk of the LHV and MHV [9, 10]. The point of this maneuver is traction, not simply division, of the ligamentum venosum. Majno et al. [9] performed this maneuver successfully in 54 (95%) of 57 cases of in-situ liver splitting or hepatic resection. Because the common trunk of the LHV and MHV is easily encircled, the MHV can also be isolated extraparenchymally. An isolated LHV or MHV serves as a goal of hepatic transection. Clamping of these veins can reduce blood loss during hepatectomy via vascular outflow control. We have routinely used Majno's maneuver in left hepatectomy or left lateral sectionectomy. Afterward, we combined this maneuver and the liver hanging maneuver.

The present technique allows liver hanging just on the left side of the MHV, and obviates the need for liver parenchymal dissection before taping. This technique is feasible and useful in left hepatectomy with the caudate lobe and without the MHV. This technique may also be useful in extended right hepatectomy or right trisectionectomy. On the other hand, left hepatectomy without the MHV and without the caudate lobe does not require the present technique. Such hepatectomy is possible if the tape is located along the ligamentum venosum with its upper end between the MHV and LHV.

The extraparenchymal isolation of the MHV can be performed safely in almost all cases, as has been mentioned. However, extraparenchymal isolation of the LHV or MHV is not indicated for cases of tumor near the roots of these veins. Furthermore, potential abnormal venous branches near the roots should be investigated by intraoperative ultrasonography. Incautious dissection between the LHV and MHV may injure these vessels. When a resistance-free dissection route cannot be found, the dissection should be abandoned, and these vessels should be encircled after dividing the liver parenchyma between the MHV and LHV.

In the liver hanging maneuver, the blind dissection between the caudate lobe and IVC carries a potential risk of inadvertent tearing of the short hepatic veins. Strict positioning and gentle advance of a Kelly clamp along the anteromedian surface of the IVC can reduce the risk. Intraoperative ultrasonography is useful for checking for abnormal short hepatic veins and as guidance during the retrohepatic dissection [4]. The liver hanging maneuver is not indicated when the tumor has invaded the retrohepatic IVC.

In conclusion, the present modified liver hanging maneuver is feasible and useful in left hepatectomy with the caudate lobe and without the MHV.

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How I Do It

Pancreatic duct holder for facilitating duct-to-mucosa pancreatojejunostomy after pancreatoduodenectomy

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KEYWORDS:
Duct-to-mucosa;
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Pancreatic duct holder;
New device

Abstract. Duct-to-mucosa pancreatojejunostomy after pancreatoduodenectomy may be technically difficult, particularly in cases in which the remnant pancreas is soft with a small main pancreatic duct. We devised a pancreatic duct holder for duct-to-mucosa pancreatojejunostomy. The holder has a cone-shaped tip. A one-third circle of the tip is cut away, which makes a slit. As the tip is inserted gently into the pancreatic duct, the duct can be adequately expanded. The holder provides a good surgical field for anastomosis. A slit of the tip allows needle insertion. The holder facilitates stitches of the jejunum also. Twelve patients underwent pancreatoduodenectomy, followed by duct-to-mucosa pancreatojejunostomy using the holder. The holder allowed 8 or more stitches in duct-to-mucosa anastomosis, even in patients with a small pancreatic duct. No patients developed prolonged pancreatic leakage or pancreatic fistula postoperatively. In conclusion, the pancreatic duct holder is a simple and useful tool for facilitating duct-to-mucosa pancreatojejunostomy.
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Pancreatoenterostomic leakage is still a common and serious complication after pancreatoduodenectomy, despite recent advances in operative techniques and postoperative care. Various methods have been used to prevent the pancreatic leakage, including duct-to-mucosa anastomosis, pancreatogastrostomy, and pancreatic duct stenting.[1–4] However, duct-to-mucosa pancreatojejunostomy may be technically difficult, particularly in cases in which the remnant pancreas is soft with a small main pancreatic duct, namely high-risk cases for pancreatic leakage or fistula.[5] We devised a pancreatic duct holder for facilitating duct-to-mucosa pancreatojejunostomy.

Surgical Technique

The pancreatic duct holder or a dilator for suture placement is constructed of metal. The holder consists of a cone-shaped tip (15-mm height, 1-mm diameter at the tip, and 6-mm diameter at the base) and a handle (Fig. 1). At the tip portion, a one-third circle of the cone is cut away, which makes a slit.

The tip of the holder is inserted gently into the pancreatic ductal lumen (Fig. 2). The pancreatic duct can be adequately expanded to 3 to 5 mm with the cone-shaped tip, even in cases with a small pancreatic duct. The holder provides a good surgical field for anastomosis. A slit of the tip allows needle insertion. Furthermore, the holder, which is inserted into a small opening of the jejunum, facilitates stitches of the jejunum also. Surgeons can avoid grasping the pancreatic duct, parenchyma, or the jejunal wall with forceps.

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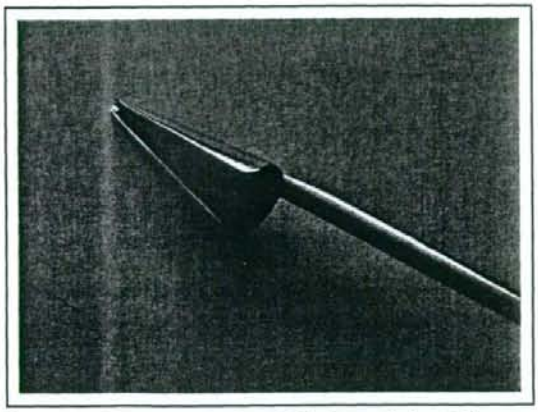

Figure 1 The pancreatic duct holder has a tip in which a one-third circle of a cone is cut away.

After pancreatoduodenectomy, pancreatojejunostomy is conducted in end-to-side anastomosis using 2 layers including duct-to-mucosa and the pancreatic parenchyma-to-the jejunal seromuscular layer[6] (Fig. 3). Duct-to-mucosa anastomosis is performed with the pancreatic duct holder, under visual magnification (2.5×). The pancreatic duct with a little pancreatic parenchyma is anastomosed to the jejunum (the mucosal layer and a small part of the seromuscular layer) using interrupted sutures (5–0 Maxon, monofilament polyglyconate; Davis & Geck, St Louis, MO). A 5F polyvinyl chloride tube (Sumitomo Bakelite, Tokyo, Japan) is inserted through the anastomosis into the pancreatic duct for partial external drainage of pancreatic juice. The opposite end of the pancreatic tube is introduced externally through the jejunal wall and the abdominal wall. Closed drains are placed anteriorly and posteriorly to the pancreatojejunal anastomosis.

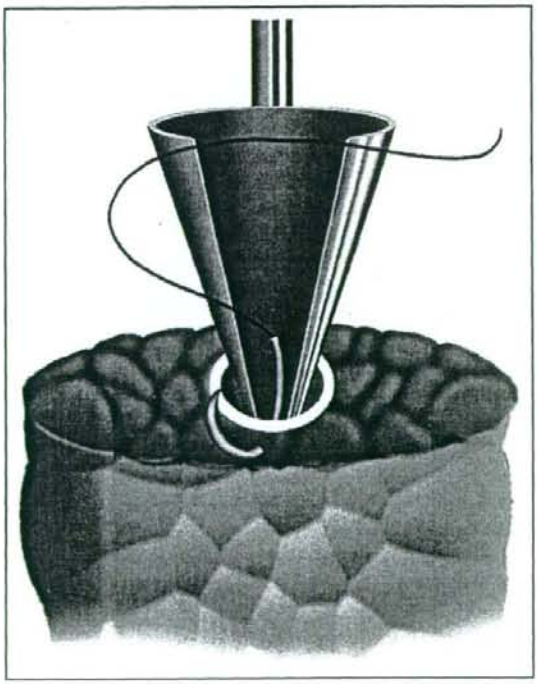

Figure 2 A pancreatic ductal stitch is placed with the pancreatic duct holder in duct-to-mucosa pancreatojejunostomy.

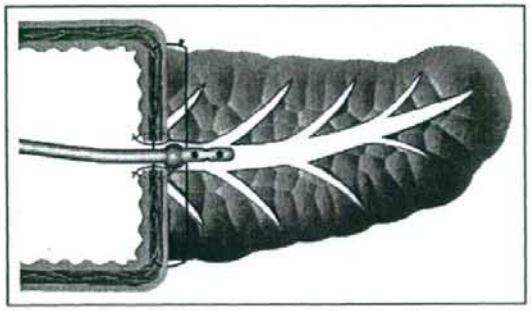

Figure 3 Pancreatojejunostomy is conducted using 2 layers including duct-to-mucosa and the pancreatic parenchyma-to-the jejunal seromuscular layer. For the latter layer, sutures penetrate the pancreatic parenchyma from the anterior surface to the posterior surface and pick up seromuscular layer of the jejunum widely, after Kakita's method.[6] A drainage tube is inserted through the anastomosis into the pancreatic duct.

Results

Between June and December 2007, 12 patients underwent conventional (n = 2) or pylorus-preserving (n = 10) pancreatoduodenectomy followed by duct-to-mucosa pancreatojejunostomy using the pancreatic duct holder. Diagnoses included bile duct carcinoma (n = 6), pancreatic head carcinoma (n = 4), and intraductal papillary mucinous neoplasm (n = 2). At surgery, the diameter of the main pancreatic duct at the cut surface measured ≤2 mm (small duct with soft pancreas) in 7 patients and >2 mm (dilated duct) in the 5 remaining patients. For duct-to-mucosa pancreatojejunostomy, 8 and 12 to 16 stitches were performed in patients with the small and dilated duct, respectively.

Postoperatively, no patients developed pancreatic fistula or prolonged (>7 days) high output (>50 mL/d) of an amylase-rich content (amylase concentration >3 times the upper limit of the normal serum amylase level) from perianastomotic peritoneal drains. The daily discharge from the pancreatic tube was 287 ± 109 mL (mean ± standard deviation).

During the postoperative follow-up period (2–9 months; median, 6 months), no patients had steatorrhea. Postoperatively, none of 10 patients who had been preoperatively nondiabetic became diabetic, but 1 of 2 diabetic patients experienced exacerbation of diabetes. Of 9 patients who

underwent a computed tomography scan or magnetic resonance imaging 3 to 9 months after surgery, none had anastomotic stenosis of pancreatojejunostomy or pancreatic ductal dilatation.

Comments

Duct-to-mucosa pancreatojejunostomy after pancreatoduodenectomy may be technically challenging in cases with a small pancreatic duct. The pancreatic ductal lumen is often collapsed after pancreatic transection. Many surgeons use fine pickup forceps, grasping the pancreatic duct, inserting it into the duct, or trying to open the ductal lumen. However, they usually fail to dilate the ductal lumen in a round shape, and the lumen becomes rather flattened. A stay suture may facilitate the subsequent stitches. Nevertheless, placement of the first stay suture itself is inevitably difficult. Stitches for a small opening of the jejunum may also be difficult because the jejunal wall is somewhat thick and sometimes inverted. Furthermore, forceps manipulation or incorrectly pulled stay sutures may cause a laceration of the pancreatic duct or parenchyma. Such manipulation may also result in inadvertent enlargement of the jejunal opening at the end of anastomotic procedure, despite an initially small incision.

The pancreatic duct holder presented in this article has several advantages. The cone-shaped tip can adequately expand the pancreatic ductal lumen as a round shape and offer excellent visualization for anastomosis. The holder is useful also for jejunal stitches. This device enables precise and fine stitches, allowing 8 or more sutures. The holder gives no damages on the pancreatic duct and parenchyma and the jejunum.

In conclusion, the pancreatic duct holder described here is a simple and useful tool for facilitating duct-to-mucosa pancreatojejunostomy.

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Adverse prognosis of epigenetic inactivation in *RUNX3* gene at 1p36 in human pancreatic cancer

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Alteration in transforming growth factor-β signalling pathway is one of the main causes of pancreatic cancer. The human runt-related transcription factor 3 gene (*RUNX3*) is an important component of this pathway. *RUNX3* locus 1p36 is commonly deleted in a variety of human cancers, including pancreatic cancer. Therefore, we examined genetic and epigenetic alterations of *RUNX3* in human pancreatic cancer. Thirty-two patients with pancreatic cancer were investigated in this study. We examined the methylation status of *RUNX3* promoter region, loss of heterozygosity (LOH) at 1p36, and conducted a mutation analysis. The results were compared with clinicopathological data. Promoter hypermethylation was detected in 20 (62.5%) of 32 pancreatic cancer tissues, confirmed by sequence of bisulphite-treated DNA. Loss of heterozygosity was detected in 11 (34.3%) of 32 pancreatic cancers. In comparison with clinicopathological data, hypermethylation showed a relation with a worse prognosis ($P=0.0143$). Hypermethylation and LOH appear to be common mechanisms for inactivation of *RUNX3* in pancreatic cancer. Therefore, *RUNX3* may be an important tumour suppressor gene related to pancreatic cancer.

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Keywords: *RUNX3*; pancreatic cancer; hypermethylation; loss of heterozygosity

Genetics and Genomics

Despite its relatively low incidence of approximately 10 cases/100 000 people, pancreatic cancer is still one of the leading causes of cancer-related death in industrialised countries including Japan. The prognosis remains poor, with an overall 5-year survival rate of less than 5% (Jemal *et al*, 2007). The pathogenesis of pancreatic ductal adenocarcinoma can be described as a step-by-step accumulation of genetic changes, such as K-ras oncogene mutations, p53, p16, and smad4 tumour suppressor gene mutations (Kern *et al*, 2002), in addition to several epigenetic alterations, which together result in self sufficiency of growth signals, insensitivity to antigrowth signals, evasion of apoptosis, angiogenesis, invasion, and metastasis (Ozawa *et al*, 2001). Recently, several reports indicated that every silencing mechanism, such as loss of heterozygosity (LOH) and mutations in a gene, or hypermethylation in its promoter region occurred in a tumour suppressor gene resulting in loss of its function in tumorigenesis (Tokumaru *et al*, 2003).

Transforming growth factor-β (TGF-β) signalling is a well-established tumour suppressor pathway in pancreatic carcinogenesis (Massagu *et al*, 2000). Smad4 is a key transcription factor in the TGF-β1 signalling pathway, and is inactivated in about 50% of pancreatic adenocarcinomas. The human runt-related transcriptional factor 3 (*RUNX3*) gene also plays important roles in the TGF-β signalling pathway. In this pathway, Smad2 and Smad3 activated by TGF-β interact with RUNX3, and induce transcriptional activation of target genes in the nucleus (Ito and Miyazono, 2003; Miyazono *et al*, 2003).

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RUNX3 induced apoptosis in epithelial cells, and the knockout mice of this gene showed hyperplasia in gastric mucosa. In addition, loss of function of RUNX3 caused by DNA hypermethylation, LOH at gene locus, and mutation correlated with the progression of primary gastric cancers (Li *et al*, 2002). RUNX3 might have the important role of TGF-β and Smad proteins in carcinogenesis. Furthermore, *RUNX3* is located on the distal portion of the short arm of human chromosome 1 (1p36), which is commonly deleted in a variety of human cancers, including pancreatic cancer (Nowak *et al*, 2005; Loukopoulos *et al*, 2007). Therefore, the genetic and epigenetic alterations in *RUNX3* may have an important role in pancreatic cancer.

The aim of our present study was to determine whether the *RUNX3* gene alteration might have a role in carcinogenesis in pancreatic cancer. We examined LOH at this gene locus in 1p36 with microdissected DNA, the DNA-methylation status by methylation-specific polymerase chain reaction (MSP) and sequencing, and the mutation of *RUNX3* by reverse transcription-polymerase chain reaction (RT-PCR) single-strand conformation polymorphism (RT-PCR-SSCP) in 32 primary pancreatic cancer tissues and corresponding noncancerous tissues. Then, we correlated these results with the clinicopathological data.

MATERIALS AND METHODS

Patients, sample collection, microdissection, and DNA preparation

Thirty-two primary pancreatic cancer tissues and corresponding noncancerous tissues were collected at Nagoya University Hospital from pancreatic cancer patients during pancreatico-duodenectomy,

distal pancreatectomy, or total pancreatectomy. All tissues were diagnosed histologically as pancreatic cancer. Written informed consent, as required by the institutional review board, was obtained from all patients. Collected samples were stored immediately in liquid nitrogen at −80°C until analysis. Genomic DNA was obtained from these samples by digestion with proteinase K, followed by phenol/chloroform extraction.

Other parts of the specimens were formalin-fixed for 24 h and processed for paraffin embedding. From each tissue block, a series of four 5-μm thick sections were cut. The first section was H&E stained for pathologic evaluation; identification of the tumour epithelia. To avoid normal cell contamination, target epithelial cells from the cancer areas were produced by laser capture microdissection using a Pixcell LCM system (Arcturus Engineering Inc., Mountain View, CA, USA). An average of 200 laser shots (30 μm shot size, 60 ms laser pulse duration, and power of 60 MW) were used for each sample. Microdissected cells were then incubated overnight at 37°C in 50 μl digestion buffer (10 mmol l^{-1} Tris-HCl (pH 8.0), 1 mmol l^{-1} EDTA, 1% Tween 20, 1 mg ml^{-1} proteinase K) and incubated at 95°C for 10 min to inactivate the proteinase K.

Microsatellite analysis

DNAs from primary pancreatic cancer tissues and corresponding noncancerous tissues were analysed for LOH study by amplification of CA repeat sequences using PCR. DNAs of pancreatic cancer epithelia were collected by the microdissection method mentioned above.

Two microsatellite markers, D1S234 and D1S247, were used. D1S234 exists at only 900 Kb on the telomeric side from the RUNX3 locus, and D1S247 is centromeric from the *RUNX3* locus. Polymerase chain reaction amplification was performed containing [α^{32}P]dCTP and 50 ng of genomic DNA. Polymerase chain reaction products were analysed on a 6% polyacrylamide gel and processed by autoradiography. Allelic loss was scored when the band intensity of one allele was decreased significantly (more than 40% reduction) in tumour DNA as compared with that in the normal DNA by using a BASS-2000 image analyzer (Fuji Photo Film Co. Ltd, Tokyo, Japan).

Methylation-specific PCR

DNA from tumour and normal specimens was subjected to bisulphite treatment. Briefly, 2 μg of DNA was denatured by NaOH and modified by sodium bisulphite. DNA samples were then purified using the Wizard purification resin (Promega Corp., Madison, WI, USA), treated again with NaOH, precipitated with ethanol, and resuspended in water. The primer pairs for the unmethylated detecting were in *RUNX3* promoter region near exon 1: S (sense, 5′-GTGGGTGGTTGTTGGGTTAGT-3′) and AS (antisense, 5′-TCCTCAACCACCACTACCACA-3′), which amplify a 138-base pair (bp) product, and those for the methylated detecting were in the same region: S (sense, 5′-CGTCGGGTTAGCGAGGTTTC-3′) and AS (antisense, 5′-GCCGCTACCGCGAAAAACGA-3′), which amplify a 120-bp product. The PCR amplification consisted of 35 cycles of 94°C for 20 s, 60°C for 20 s, and 72°C for 15 s, after the initial denaturation step (94°C for 5 min). Each PCR product was loaded directly onto 2% agarose gels, stained with ethidium bromide, and visualised under UV illumination.

Sequence analysis

Genomic bisulphite-treated DNA of primary pancreatic cancer tissues was sequenced. Polymerase chain reaction was performed in methylated cases. The primer pairs for sequence were in RUNX3 promoter region near exon1: S (sense, 5′-GTTTAGGTAGTAGGGATAGTT-3′) and AS (antisense, 5′-CTATTCTCTCCCATCTTACC-3′), which amplify a 388-bp product. The PCR amplification consisted of 36 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 30 s, after the initial denaturation step (94°C for 5 min). Polymerase chain reaction products were purified directly using the QIA quick Gel Extraction Kit (QIAGEN, Hilden, Germany). Purified DNA fragments were subcloned into TA cloning vector (Invitrogen™, Carlsbad, CA, USA). Six cloning samples were picked out from one methylated tumour tissue. Each cloning DNA was mixed with 3 μl of specific primer (M13), 4 μl of Cycle Sequence Mix (ABI PRISM Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA, USA). Samples were subjected to the following cycling conditions: 95°C for 30 s; 25 cycles of 95°C for 15 s, 50°C for 15 s, and 60°C for 4 min followed by purification by ethanol precipitation. Sequence analysis was carried out using an Applied Biosystems ABI310, and sequence electropherograms were generated by ABI Sequence Analysis 3.0.

RT-PCR-SSCP

Polymerase chain reaction amplification using random-primed cDNA of 32 primary pancreatic cancer tissues was performed using oligonucleotide primers in the presence of [α^{32}P]dCTP, followed by electrophoretic separation on 6% nondenaturing polyacrylamide gels both in the presence of 5% glycerol at room temperature and in its absence at 4°C. RUNX3 ORF (1248-bp) is divided into four overlapped fragments and each fragment was amplified. The primer pairs used for *RUNX3* mutation were S1 (sense, 5′-GCCGCTGTTATGCGTATTCC-3′) and AS1 (antisense, 5′-CTCAGCGGAGTAGTTCTCGT-3′), amplifying a 370-bp fragment; S2 (sense, 5′-GTGACTGTGATGGCAGGCAA-3′) and AS2 (antisense, 5′-GTTCCGAGGTGCCTTGGATT-3′), amplifying a 398-bp fragment; S3 (sense, 5′-ACAAGCCACTTCAGCAGCCA-3′) and AS3 (antisense, 5-GAGAACTGGTAGGAGCCAGA-3′), amplifying a 368-bp fragment; S4 (sense, 5′-CTACCACCTCTACTACGGGA-3′) and AS4 (antisense, 5′-CCCATCACTGGTCTTGAAGG-3′), amplifying a 326-bp fragment. The PCR amplification consisted of 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, after the initial denaturation step (94°C for 5 min) in F1–R1 and in the presence of 10% dimethylsulphoxide (F2–R2, F3–R3, F4–R4).

Statistical analysis

The correlation between the methylation status of RUNX3 mRNA and clinicopathological data was analysed by Fisher's exact test or χ^2 test for independence. Overall survival rates were calculated using the Kaplan–Meier method, and difference in survival curves was analysed using the log-rank test. Independent prognostic factors were identified by multivariate analysis using the Cox proportional hazards regression model. Data are expressed as mean ± s.d. Statistical significance was considered as $P<0.05$.

RESULTS

Microsatellite analysis of *RUNX3*

We first examined DNA samples obtained by microdissection from the 32 primary pancreatic cancer tissues and corresponding noncancerous tissues for LOH using two microsatellite markers, D1S234 and D1S247, which are close to the RUNX3 locus. D1S234 is telomeric and D1S247 is centromeric to the locus. Allelic imbalance in one or two markers was observed in 11 (34.3%) of the 32 cases (Figure 1). We judged the 11 cases as having an LOH at the locus. The results are summarised in Table 1. No cases evidenced microsatellite instability in this study. Two cases proved noninformative from using the two markers.

Hypermethylation of *RUNX3* promoter region in pancreatic cancer

To investigate whether the gene silencing was due to hypermethylation of *RUNX3*, MSP was performed in the 32 primary pancreatic

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