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Pancreatic parenchymal injection of ethanol and octreotide to induce focal pancreatic fibrosis in rats: Strategies to eliminate postoperative pancreatic fistula

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ABSTRACT

Background: Postoperative pancreatic fistula (POPF) is more likely to occur in a soft pancreas compared to a hard pancreas in which fibrosis has progressed. There is almost no leakage at the anastomosis site or cut surface of a hard pancreas. The aim of this study was to induce localized fibrosis at the cut surface of the pancreas in a rat model.

Methods: Thirty-six rats were divided into three groups (group S: normal saline group; group E: ethanol group; and group O: octreotide group). Each rat was directly injected with a particular compound at the duodenal lobe of the pancreatic parenchyma. Each group was divided into three subgroups according to the time of post-injection sacrifice (1, 2, or 4 weeks). The hardness, suture holding capacity (SHC), and histological fibrosis grade of each pancreas were measured.

Results: The hardness, SHC, and fibrosis grade of groups E and O were increased at week 1, with greater increases in group E (all $P < 0.001$). In a subgroup comparison, the hardness, SHC, and fibrosis grade of group E tended to decrease gradually over time, with no regular pattern evident in group O. A comparison between the injected site (duodenal lobe) and non-injected site (splenic lobe) of the pancreas revealed increases in the three parameters of group E only in the duodenal lobe, with increases in group O at both the duodenal and splenic lobes.

Conclusions: Parenchymal injection of ethanol and octreotide increased pancreatic fibrosis. Unlike octreotide, ethanol provoked localized fibrosis that was maintained over time. It is expected that ethanol injection could eliminate POPF during pancreatic surgery.

Introduction

Postoperative leakage of pancreatic juice is an important leading cause of mortality in pancreatic surgery. It is associated with infection and bleeding, both of which can be life-threatening. The pancreatic fistula rate after pancreaticoduodenectomy (PD) is as high as 20% [1,2]. Most postoperative pancreatic fistulas (POPF) occur at the pancreaticoenteric anastomosis site or pancreatic stump after PD. Many studies have sought to achieve secure pancreaticoenteric anastomosis and optimal handling of the pancreatic cut surface. These studies included improvements in anastomotic methods of the remnant pancreas, such as invagination pancreaticojejunostomy, duct-to-mucosa pancreaticojejunostomy, binding techniques, intraoperative insertion of a pancreatic duct stent at the pancreaticoenteric anastomosis site, and many other modified procedures [3–8]. Other efforts have focused on handling cut surfaces of the pancreas after PD, such as using a linear stapler, fibrin glue, or a bioabsorbable or non-absorbable re-enforced linear stapler [9,10]. However, pancreatic leakage has not been solved.

Many experts agree that the histopathological features of the pancreas, specifically the texture of the remaining pancreas, are key factors for the development of POPF [11–13]. The possibility of POPF occurrence is much higher in a soft pancreas compared to a hard pancreas. Pancreatic hardening occurs mainly due to the fibrosis of the pancreatic parenchyma. When fibrosis forms instead of the duct structure in the pancreatic parenchyma, the hardness of the pancreas increases and the suture holding capacity (SHC) increases, which results in decreased secretion of pancreatic juice. Thus, the texture of the remnant pancreatic tissue that is anastomosed with other viscera (stomach or small bowel) is an impor-

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tant factor for reducing complications after surgery. However, there have been few studies on the texture of pancreatic tissue [11].

If preoperative focal fibrosis at the pancreas anastomosis site is artificially formed safely to preserve pancreatic function, the pancreaticoenteric anastomosis suture will be stable and the leakage of pancreatic juice could be eliminated. For this reason, we investigated an effective modality for investigating focal pancreatic fibrosis.

Methods

Experiments were performed in the animal laboratory of Seoul St. Mary's Hospital with approval of the ethics committee and under supervision from the institute's veterinarians.

Animal grouping

Thirty-eight male 8- to 12-week-old Sprague–Dawley rats were purchased from Orient Bio (Gyeonggi, Korea) and were housed in a pathogen-free facility with free access to water and food. The rats were divided into three groups ($n = 12$), with an additional two rats allocated to a sham group. Rats in the sham group received no treatment. Rats in group S received 1 mL of a 0.9% normal saline injection directly into the pancreas. Rats in group E received 1 mL of medical-grade 90% ethanol. Rats in group O received 1 mL of a solution containing 100 μg octreotide (Sandostatin, Novartis, Switzerland). Four rats from each group were sacrificed at week 1, 2, and 4 post-injection.

Injection technique

Each rat was placed in the anesthesia induction chamber with isoflurane and oxygen mixed gas. The oxygen flowmeter was adjusted to 0.8–1.5 L/min, and the isoflurane vaporizer was adjusted to 3%–5%. After induction of anesthesia, the rat was transported and fastened to an operation bench with the ventral side facing up. During the operation, inhaled anesthesia was maintained through a facemask with a flow rate of 0.4–0.8 L/min of 2% isoflurane vapor. After skin shaving and asepsis using 70% alcohol, a ventral skin incision approximately 3 cm long was made on the abdominal wall, and the wound was retracted by a self-retractor before the pancreas was carefully exposed. Materials were injected directly at the parenchyma of the pancreas using a 1 mL syringe equipped with a 24-gauge needle at different sites of the duodenal lobe of the pancreas with a thin puncture of pancreatic capsule to evenly distribute the injected material (Fig. 1). Abdominal wound closure was performed in two stages. The peritoneum and muscle layer were closed with Vicryl™ #3–0 (Polyglactin 910; Ethicon, Somerville, NJ, USA) interrupted sutures, and the skin was closed with Dermalon™ #3–0 (Nylon; Ethicon) interrupted suture. To avoid modifying the tissue response to the injected material, nonsteroidal anti-inflammatory drugs, opioids, analgesics, and prophylactic antibiotics were not used in this study.

On the day of pancreas harvest, the animals were reanesthetized using the same protocol. Laparotomy with a 3-cm midline incision was performed, and the pancreas was harvested along with the adherent duodenum and spleen. The pancreatic tissues were divided into two parts: the duodenal lobe and the splenic lobe distant from the injection site. All rats were sacrificed through natural bleeding during the harvest procedure.

Measurements of pancreatic tissue

Hardness and SHC were measured, and a histological examination was conducted to evaluate focal pancreatic fibrosis. Hardness and SHC were measured immediately after pancreas was harvested by one investigator who received the tissue specimens in random order and was blinded to the origin of the tissues. The histology was assessed by an experienced pathologist in our hospital.

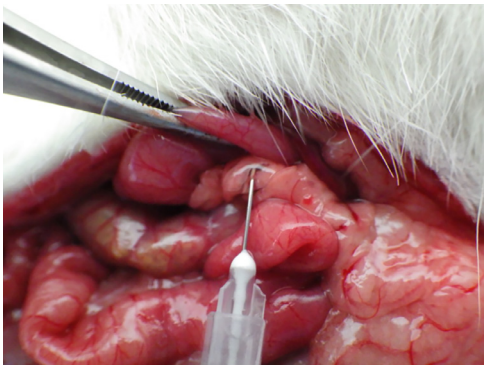

Fig. 1. Reagent was injected into the pancreatic parenchyma directly.

Hardness was measured using a Check-line DD-100 durometer (Electromatic Equipment Co., Cedarhurst, NY, USA). The durometer was placed on the operating stand (OS-4H; Paul N. Gardner Company, Inc., Pompano Beach, FL, USA) to provide a convenient and accurate way to perform repeated hardness tests and the correct testing pressure as well as to eliminate human error. The specimen was positioned on the operating stand table as shown in Fig. 2A. Three measurements were taken by placing the working face of the durometer on the exact area of the specimen, with results expressed as the mean value.

Immediately after the durometer measurement, SHC was measured using a model DS2-5N Newton dynamometer (Optech, Shanghai, China) (1 N = 101.97 gf) (Fig. 2B). A straight cut was performed through the specimen and a 10 × 10 mm piece of pancreas was excised. In the SHC test, the single-pass suture was made with 6–0 prolene (Polypropylene; Ethicon) on a tapered needle 3 mm from the edge of the specimen (Fig. 2C). The specimens were sutured from both sides. One side was fixed at the clamp, and the other side was knotted to connect with the Newton dynamometer. The dynamometer with the specimen was placed on the operating stand with the durometer, and an SHC test was performed until the suture tore through the tissue (pull-out) at a rate of 0.02 mm/s. The test was performed three times in each specimen.

After measuring the hardness and SHC, the rest of the pancreatic tissue was fixed in formalin for histology. Masson's trichrome staining (Sigma-Aldrich, Inc., St. Louis, MO, USA) was performed to evaluate the degree of pancreatic tissue fibrosis using a microwave procedure. To evaluate pancreatic fibrosis, a histopathological fibrosis parameter was adopted. The histopathological measurement length from actual measurement of interlobular and intralobular collagen fibers was calculated using the average length of 10 points of view. The grade of pancreatic fibrosis was evaluated according to a four-grade scoring system [14], including normal pancreatic parenchyma and no fibrotic changes (grade 0), mild fibrosis with thickening of periductal fibrosis (grade 1), moderate fibrosis with marked sclerosis of interlobular septa or intralobular sclerosis with no evidence of architectural changes (grade 2), and severe fibrosis with detection of architectural destruction or acinar cell atrophy (grade 3) (Fig. 3).

Statistical analysis

Statistical analyses were performed using SPSS for Mac software version 13.0 (SPSS Inc., Chicago, IL, USA). Continuous data are presented as the mean ± standard deviation (SD). For continuous data, overall differences were tested by Student's *t* test and ANOVA. Multiple comparison tests were used for further analysis.