Int Surg 2023;**107:**15–22
DOI: 10.9738/INTSURG-D-18-00040.1

Superiority of Somatostatin Analog in Comparison With Drugs for Treating Pancreatic Fistula in Rats

Yusuke Kawakami, Tomohiko Adachi, Shinichiro Ono, Naoki Kuroshima, Hajime Imamura, Toshiyuki Adachi, Takanori Hirayama, Amane Kitasato, Akihiko Soyama, Masaaki Hidaka, Mitsuhisa Takatsuki, Tamotsu Kuroki, and Susumu Eguchi

Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Objective: This study aims to identify the most effective individual drug in an established triple-drug therapy (TDT) using a postoperative pancreatic fistula (POPF) rat model.

Summary of background data: POPF is the major complication of pancreatectomy that causes intraperitoneal abscess, sepsis, and pseudoaneurysm rupture, all of which may prolong hospital stays and cause potentially serious events or death. We previously demonstrated that TDT with a somatostatin analog, gabexate mesilate, and imipenem/cilastatin effectively prevents POPF, especially in high-risk patients.

Methods: POPF-induced rats were killed on postoperative day 3 after control (C), gabexate mesilate (G), imipenem/cilastatin (I), and somatostatin analog (S) treatments. Levels of serum amylase and lipase, or ascitic amylase and lipase were measured. Intraperitoneal adhesion between the abdominal wall and pancreas and pancreatic inflammation were evaluated.

Results: Serum amylase levels did not significantly differ among the groups. Serum lipase level was significantly higher in group I than in the other groups ($P < 0.01$). Both ascitic amylase and lipase levels were significantly lower in group S than in the other groups ($P < 0.01$). Median inflammation scores were significantly lower in groups G, I, and S than in group C ($P < 0.01$). Moreover, adhesion score was lower in group S than in the other groups (groups C, G, I, and S with scores 3, 2, 3, and 1, respectively, $P < 0.01$).

Conclusion: Among the 3 drugs, the somatostatin analog was the most effective against POPF.

Key words: Pancreatic fistula – Triple-drug therapy – Somatostatin analog

Corresponding author: Susumu Eguchi, Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
Tel.: +81 95819 7316; Fax: +81 95819 7319; E-mail: sueguchi@nagasaki-u.ac.jp

Pancreatectomies have become much safer because of improvements in surgical techniques and devices,[1–5] but postpancreatectomy complications still occur at a rate of 30 to 50%.[6] Postoperative pancreatic fistula (POPF), which is the leakage of pancreatic exocrine secretions, is the major complication of pancreatectomy, with reported incidence rates of 10% or 28%.[7,8] POPF causes several additional complications, such as intraperitoneal abscess, sepsis, and pseudoaneurysm rupture, which all result in prolonged hospital stays, potentially serious events, or death.[9,10] Because of its severity, preventing POPF is challenging but critical to surgeons. Numerous studies have been performed on POPF,[11–13] but those that aimed to investigate the prevention of POPF have only reached a consensus on the need.[14,15] In addition, no optimal drug treatment is currently available for POPF.

We previously conducted a clinical research study for the prevention of POPF after distal pancreatectomy,[16] and we demonstrated that triple-drug therapy (TDT, with a somatostatin analog, gabexate mesilate, and imipenem/cilastatin) effectively prevents POPF, especially in high-risk patients. In addition, we experimentally confirmed the efficacy of TDT for POPF[17] using the rat POPF model we previously reported.[18] In this study, we employed all drugs that could be assumed to be effective because POPF is a complication that can become fatal, and it would be useful to know beforehand the superiority of each drug in this TDT for POPF, so that some drugs can be eliminated in therapy under certain circumstances. Therefore, the aim of this study was to identify the individual drug with the greatest efficacy for treating POPF.

Materials and Methods

Animals

Eight-week-old male Sprague-Dawley rats (Charles River Laboratories Japan Inc, Yokohama, Japan) were used for the experiments. Each rat weighed 260 to 280 g and was housed in a plastic cage and provided standard rat chow and water at the Laboratory Animal Center for Biochemical Research at Nagasaki University Graduate School of Biomedical Sciences. All animal protocols were approved by the Animal Experimentation Committee of Nagasaki University.

Rat model

The surgical procedure used to establish the POPF rat model was previously reported.[18] The drugs used in this experiment were the same as those used in the TDT experiment.[17] In brief, Sprague-Dawley rats were divided into the following 4 groups: control (C), gabexate mesilate (G), imipenem/cilastatin (I), and somatostatin analog (S) groups. Group C was administered normal saline, and each agent was administered to the respective group at the maximum doses for human treatment, which were 10 and 16 $mg \cdot kg^{-1} \cdot day^{-1}$ for gabexate mesilate and imipenem/cilastatin, respectively, and 5 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for the somatostatin analog. These doses were proven to be usable and comparable in the rat by the TDT experiment.[17] The drugs were administered to the rats using an osmotic pump (model 2ML1, Alzet, Cupertino, California) similar to that used in our previous experiment,[17] and the pump was filled with each drug and then implanted into the subcutaneous space on the backs of the rats, simultaneously. There were 8 rats in each group, which were killed on postoperative day (POD) 3.

Data sampling

Blood samples were collected from the inferior vena cava of the rats on PODs 0 and 3. The abdominal space of each rat was irrigated with 3 mL of normal saline, and then ascitic samples were collected and centrifuged, and the supernatants were collected on PODs 0 and 3. All samples were stored in the freezer at –80°C until analysis. The levels of serum amylase, lipase, and leukocytes, and ascitic amylase, lipase, interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) on POD 3 were measured. The cytokine levels were measured using an enzyme-linked immunosorbent assay. When the rats were killed on POD 3, relaparotomy was also performed to remove the pancreas to evaluate the degree of inflammation.

Evaluation of intra-abdominal adhesion

We evaluated the extent of adhesion in all rats as a measure of the degree of POPF on POD 3. Intra-abdominal adhesion could occur as a result of inflammation activity of the peritoneal macrophage, and the extent of POPF was in proportion with its intensity.[19] The adhesion was scored in accordance with the scale used in a previous report[17] as follows: 0 = no adhesion; 1 = adhesion that could be separated by blunt dissection; 2 = adhesion that

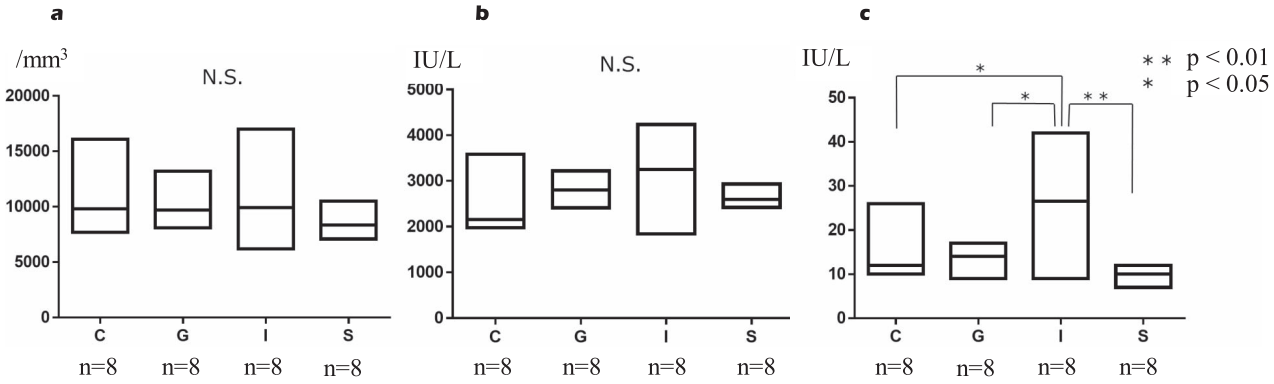

Fig. 1 (a) Serum WBC count in rat samples on POD 3. Serum (b) amylase and (c) lipase levels.

could be separated by sharp dissection; and 3 = adhesion that could not be dissected.

Pathologic examination

The pancreases that were removed after euthanasia were fixed in formalin and stained with hematoxylin and eosin; 3 pancreatic tissue sections were randomly selected, and their inflammation levels were histologically scored and summed using the following defined scale: grade 0 = no inflammation, or 1 small foci of monocular cells was present without any disruption of the cellular architecture; grade 1 = presence of <5% neutrophilic or lymphoplasmacytic inflammation; grade 2 = neutrophilic or lymphoplasmacytic inflammation between 5% and 50%; grade 3 = presence of >50% neutrophilic or lymphoplasmacytic inflammation; and grade 4 = pancreatic tissue necrosis or peripancreatic necrosis/steatitis.[20]

Statistical analysis

The continuous data are expressed as the mean ± SD and were compared using a 1-way analysis of variance. We assigned statistical significance at $P < 0.05$, and the GraphPad Prism 6 program was used for statistical analysis (GraphPad Software Inc, La Jolla, California).

Results

White blood cell (WBC) count and serum amylase and lipase levels in the rats on POD 3 are shown in Fig. 1. The mean WBC counts in groups C, G, I, and S were 9800 (7700–16,100), 9700 (8100–13,200), 9800 (6200–17,000), and 8300 (7100–10,500) cells/μL, respectively. There was no significant difference among the 4 groups. Figure 1b shows that the mean serum levels of amylase in groups C, G, I, and S were 2158 IU/L (1973–3581 IU/L), 2799 IU/L (2409–3128 IU/L), 3249 IU/L (1844–3886 IU/L), and 2595 IU/L (2418–2931 IU/L), respectively. There was also no significant difference among the 4 groups. Figure 1c shows the result of the serum lipase analysis on POD 3, and the levels in groups C, G, I, and S were 12 IU/L (10–26 IU/L), 14 IU/L (9–17 IU/L), 26 IU/L (9–32 IU/L), and 10 IU/L (7–12 IU/L), respectively. There was a significant difference between group I and the other 3 groups.

The levels of amylase, lipase, IL-6, and TNF-α in the ascitic samples are shown in Fig. 2, whereas Fig. 2a shows the ascitic amylase levels on POD3. The levels in groups C, G, I, and S were 2746 IU/L (1173–9133 IU/L), 1998 (905–4407 IU/L), 5492 IU/L (276–7551 IU/L), and 471 IU/L (130–719 IU/L), respectively. There were significant differences between groups C and S, and groups I and S. Figure 2b shows that the ascitic lipase levels in the rats in group C, G, I, and S were 43 IU/L (13–64 IU/L), 80 IU/L (6–277 IU/L), 64 IU/L (7–94 IU/L), and 10 IU/L (7–12 IU/L), respectively. There were significant differences between groups G and S. Figure 2c shows that the ascitic IL-6 levels in group G, I, and S were 2733 pg/mL (2363–2867 pg/mL), 1069 pg/mL (978–1118 pg/mL), and 92 pg/mL (43–117 pg/mL), respectively. There were significant differences between groups G and I, groups G and S, and groups I and S. Figure 2d shows that the ascitic TNF-α levels in group G, I, and S were 7.8 pg/mL (3.7–14.5 pg/mL), 13.4 pg/mL (11.6–16.9 pg/mL), and 1.2 pg/mL (0.0–7.3 pg/mL), respectively, and there were significant differences between groups G and I, groups G and S, and groups I and S.

The grading of adhesions around the pancreas is shown in Fig. 3, and the scores in each group are

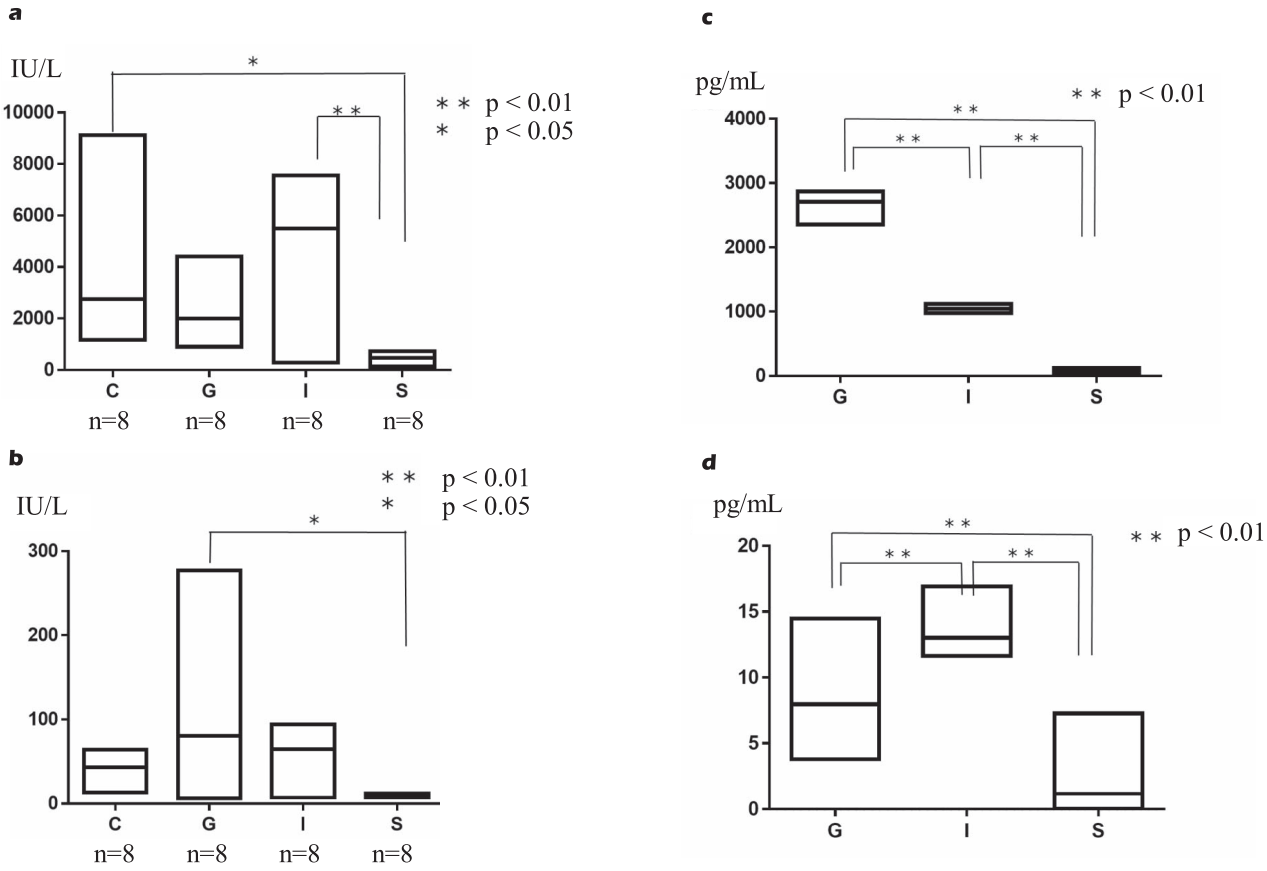

Fig. 2 (a) Ascitic amylase levels in rat samples on POD 3. Ascitic levels of (b) lipase, (c) IL-6, and (d) TNF-α. In the evaluation of IL-6 and TNF-α, control group was not set.

shown in Fig. 4. The median adhesion scores were 3 (1–3), 2 (2–3), 3 (1–3), and 1 (0–1) in groups C, G, I, and S, respectively, and the adhesions in group S were significantly milder than those in the other groups.

The histologic scoring of the pancreatic inflammation in this study is shown in Fig. 5, and the inflammation scores of the pancreatic parenchyma are shown in Fig. 6. The median inflammation scores were 9 (8–11), 5 (4–8), 6 (4–7), and 6 (4–7) in groups C, G, I, and S, respectively. Furthermore, there were significant differences between group C and the other groups.

Discussion

We previously evaluated the clinical efficacy of a TDT in preventing POPF,[16] and subsequently confirmed its efficacy as a treatment for POPF using a rat model.[17] Based on the hypothesis that a TDT would be a good treatment strategy, every available relevant drug should be investigated for the conservative treatment of POPF. This is because the clinical severity of POPF has serious implications, and therefore it needs to be prevented. However, whether all 3 drugs are necessary for the efficacy of the cotreatment, and which drug has superior therapeutic activity against POPF have not been studied. Therefore, the present study was conducted to clarify this, and the results revealed that the somatostatin analog was the most effective for treating POPF.

Some clinical trials have reported controversial findings on the prophylactic efficacy of the somatostatin analog in POPF.[21–26] In contrast, gabexate mesilate has been reported to suppress the activation of pancreatic enzymes after pancreatectomy, and IT has been used in clinical trials.[27] In treating acute necrotizing pancreatitis, the Japan Guidelines for the Management of Acute Pancreatitis recommend the use of proteolytic enzyme inhibitors.[28] Imipenem/cilastatin is an antibiotic that has good

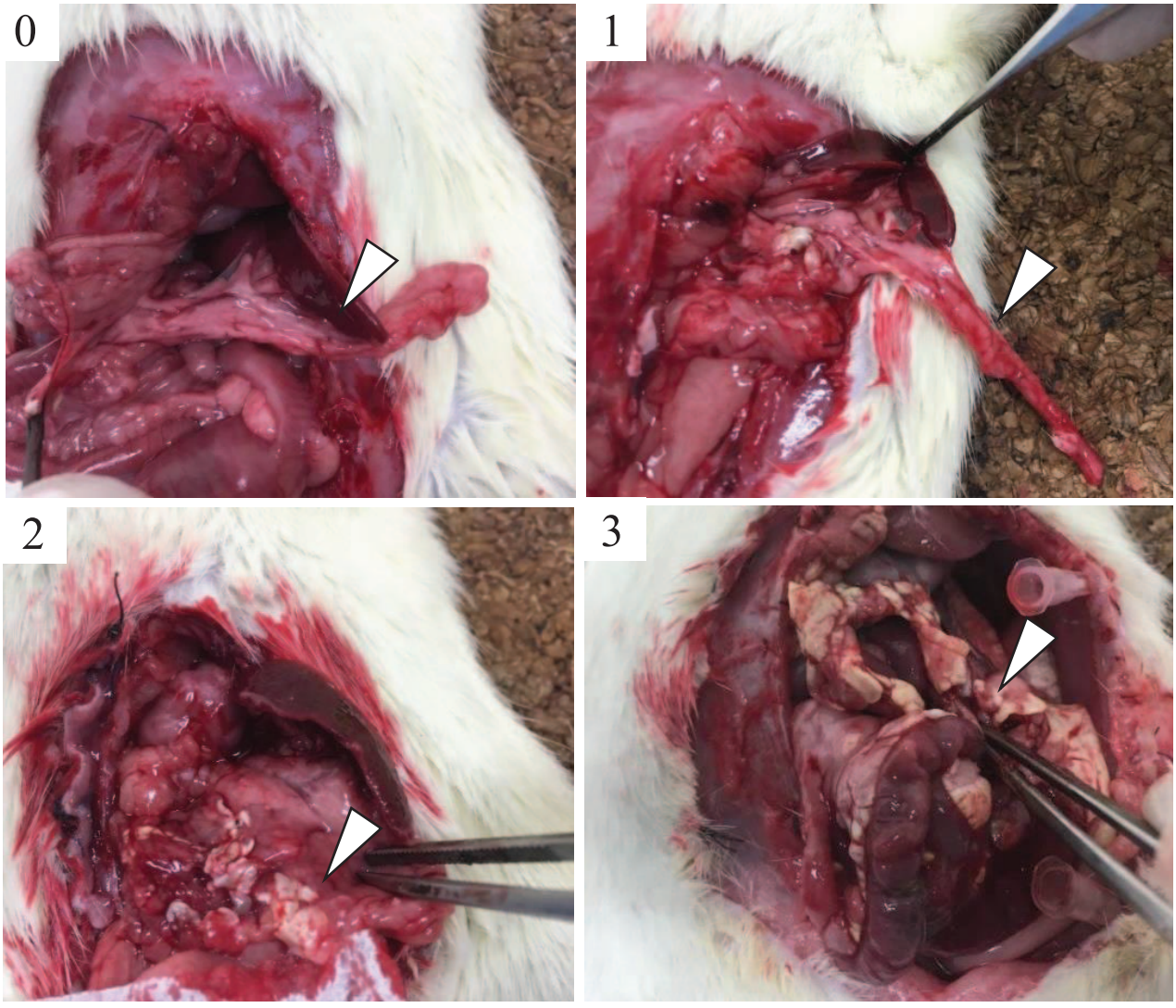

Fig. 3 Grading of adhesions around the pancreas was evaluated based on the following macroscopic findings: 0 = no adhesion, 1 = adhesion that could be separated by blunt dissection, 2 = adhesion that could be separated by sharp dissection, and 3 = adhesion that could not be separated. White arrowhead indicates the pancreas.

transferability properties in the pancreas, and IT is the only antibiotic shown to prevent infection and decrease the mortality rate of patients with necrotizing pancreatitis.[29] Infectious complications often occur secondary to severe acute pancreatitis, and most bacteria are sensitive to imipenem/cilastatin.[30] Therefore, this agent could be expected to decrease extrapancreatic and pancreatic infections and might be useful for preventing POPF.[30]

To date, the search for an appropriate therapeutic agent for POPF has proven that the closure rate of pancreatic fistula using somatostatin or octreotide, a somatostatin analog, is higher than that observed with no drug use.[31] These agents reduced pancreatic fistulas and could be assumed to have the potential for use as therapeutic agents for POPF. Furthermore, it has also been proven that using a somatostatin analog could hasten the closure of pancreatic fistulas.[32]

One of the differences between somatostatin and octreotide is their circulating half-lives. Somatostatin has a short pharmacologic circulating half-life of <3 minutes,[33] whereas its analog octreotide has a slightly longer half-life of between 1 to 2.5 hours, and therefore can be administered subcutaneously and intravenously.[34] On the other hand, pasireotide, which has a much greater binding affinity for somatostatin receptor subtypes 1, 3, and 5 than

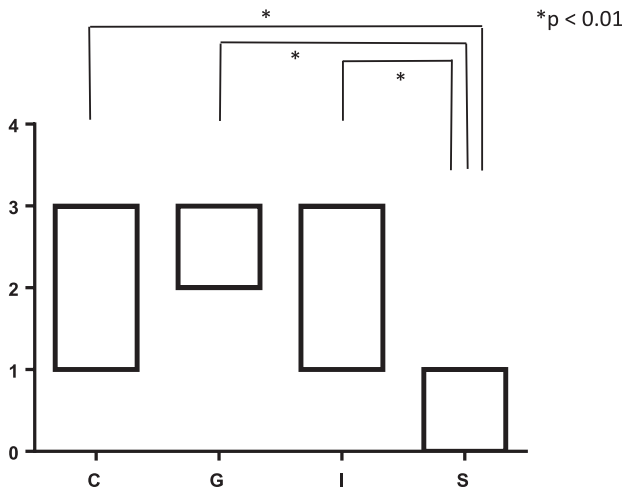

Fig. 4 Comparison of adhesion scores around the pancreas between each group.

octreotide does, has a half-life of approximately 11 hours.[34] Therefore, it could be administered subcutaneously twice a day for 1 week perioperatively to prevent POPF.[34] Somatostatin analogs are suggested to require administration via a continuous dosing pump, and our use of this strategy might have prolonged the actual duration of the effect of the drug and influenced the outcomes.

In the present study, there was no significant difference between the serum amylase levels in each group on POD 3. Pancreatic amylase is secreted from the acinar cell into the abdominal cavity in our POPF rat model. As a prerequisite, our rat POPF model was established by considering that the ascitic amylase level naturally returns to normal on POD 7.[18] This POPF model could be considered to involve reactions in the local environment, but not systemic reactions. In addition, our model does not involve acute pancreatitis in which various

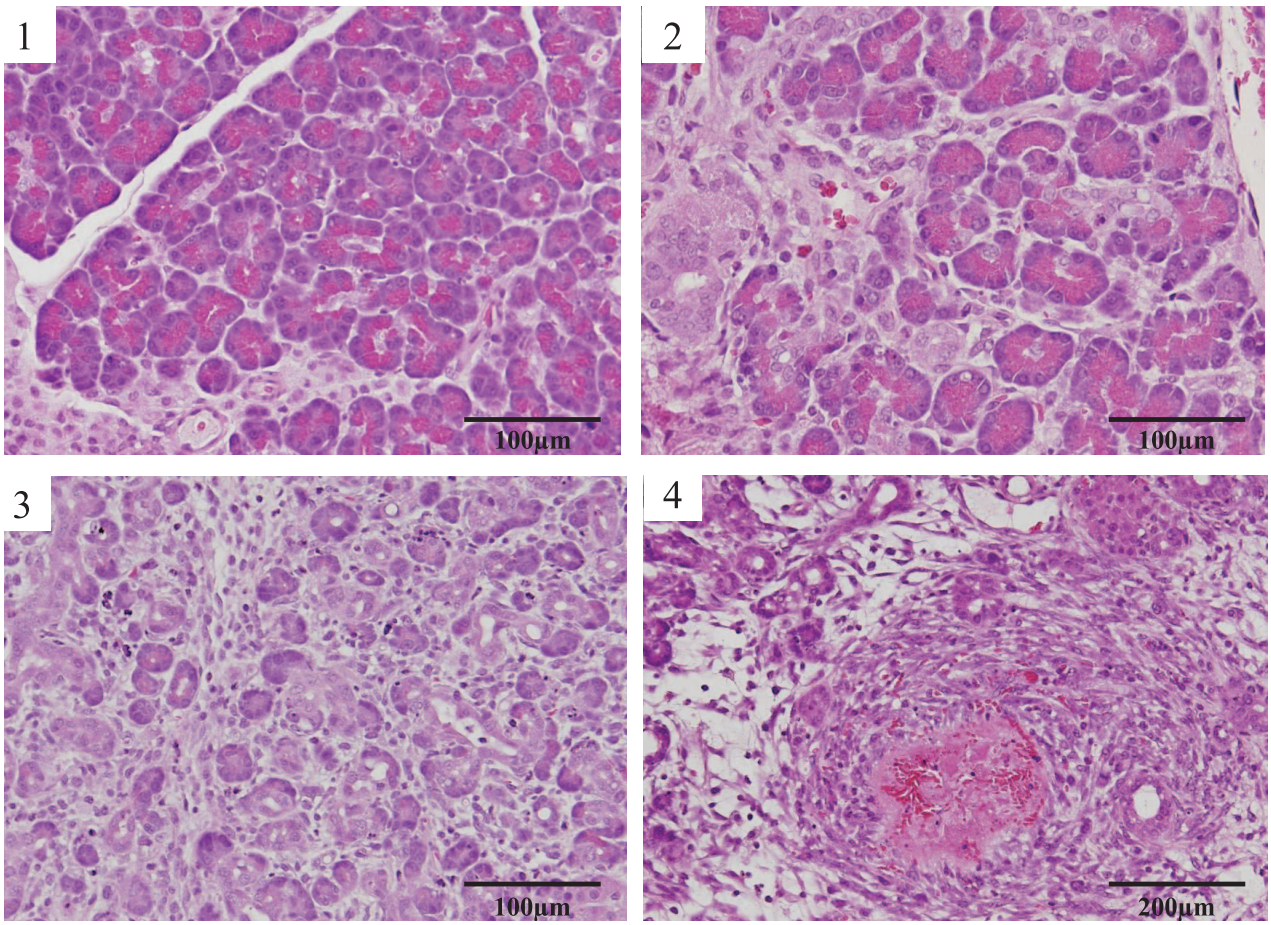

Fig. 5 Histologic scoring of pancreatic parenchyma inflammation. Scale: (1) <5%, (2) 5% to 50%, and (3) >50% neutrophilic or lymphoplasmacytic inflammation under high-power magnification, and (4) pancreatic tissue necrosis, peripancreatic necrosis, or steatitis.

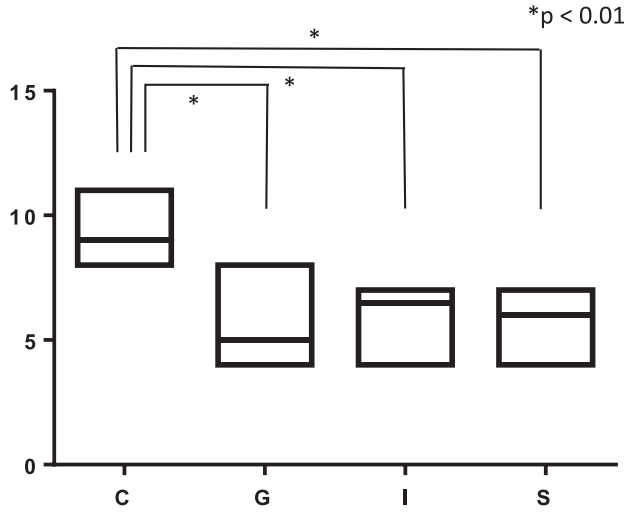

Fig. 6 Comparison of inflammation score of pancreatic parenchyma.

inflammatory mediators are released as part of the pathophysiology. Serum amylase levels were not elevated, and therefore the effectiveness of gabexate mesilate, which suppresses the activation of pancreatic enzymes, did not appear to be a contributing factor.[29,35] Only serum lipase level in group I was elevated. Although the mechanism was unknown, it might influence some adverse effect to the acinar cell, and lipase would ooze out.

The grade of the pancreatic fistula observed in group S estimated by the intraperitoneal inflammation score significantly decreased whereas both amylase and lipase levels in the ascites of group S on POD 3 were significantly lower than those of the other groups. The results revealed that the levels of IL-6 and TNF-α in group S were significantly lower than they were in the other groups, which might indicate the role of peritoneal macrophages in the local inflammatory response.[19]

The adhesion score in group S was also found to be the lowest among all the groups. When POPF occurs in the abdominal cavity, peritoneal macrophages produce proinflammatory cytokines, such as IL-6 and TNF-α, and the grade of intraperitoneal inflammation is proportional to the cytokine levels in the ascites.[19]

There was no significant difference among groups G, I, and S in the inflammation score of the pancreatic tissue. Only group C exhibited a significantly increased pancreatic inflammation score. Gabexate mesilate inhibits proteolytic enzymes,[28] imipenem/cilastatin could be expected to reduce pancreatic infection,[30] and the somatostatin analog, octreotide, inhibits pancreatic exocrine and endocrine activities.[33] These findings indicate that the 3 drugs have the potential to suppress inflammation and contribute to the treatment of POPF to some extent, although their mechanisms were not investigated in this study. Therefore, our study indirectly proved that cotreatment with the 3 drugs is a reasonable strategy, and we evaluated the superiority of the efficacy of each drug in the present study.

In conclusion, among the 3 drugs in our established TDT for POPF, the somatostatin analog was experimentally proven to be the most effective, whereas the other drugs also contributed to treating POPF by suppressing the pancreatic inflammation. These results will aid surgeons in treating and preventing POPFs.

References

1. Kuroki T, Tajima Y, Kanematsu T. Surgical management for the prevention of pancreatic fistula following distal pancreatectomy. *J Hepatobiliary Pancreat Surg* 2005;**12**(4):283–285
2. Kawai M, Tani M, Yamaue H. Transection using bipolar scissors reduces pancreatic fistula after distal pancreatectomy. *J Hepatobiliary Pancreat Surg* 2008;**15**(4):366–372
3. Suc B, Msika S, Fingerhut A, Fourtanier G, Hay JM, Holmières F. Temporary fibrin glue occlusion of the main pancreatic duct in the prevention of intra-abdominal complications after pancreatic resection: prospective randomized trial. *J Ann Surg* 2003;**237**(1):57–65
4. Misawa T, Shiba H, Usuba T, Nojiri T, Uwagawa T, Ishida Y. Safe and quick distal pancreatectomy using a staggered six-row stapler. *Am Surg* 2008;**195**(1):115–118
5. Knaebel HP, Diener MK, Wente MN, Büchler MW, Seiler CM. Systemic review and meta-analysis of technique for closure of the pancreatic remnant after distal pancreatectomy. *Br J Surg* 2005;**92**(5):539–546
6. Bassi C, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J. Postoperative pancreatic fistula: an international study group (ISGPF) definition. *Surgery* 2005;**138**(1):8–13
7. Butturini G, Daskalaki D, Molinari E, Scopelliti F, Casarotto A, Bassi C. Pancreatic fistula: definition and current problems. *J Hepatobiliary Pancreat Surg* 2008;**15**(3):247–251
8. Lee JH, Hwang DW, Lee SY, Hwang JW, Song DK. Clinical features and management of pseudoaneurysmal bleeding after pancreatoduodenectomy. *Am Surg* 2012;**78**(3):309–317
9. van Berge HMI, de Wit LT, van Gulik TM, Obertop H, Gouma DJ. Incidence, risk factors, and treatment of pancreatic leakage after pancreaticoduodenectomy: drainage versus resection of the pancreatic remnant. *J Am Coll Surg* 1997;**185**(1):18–24
10. Mech K, Wysocki L, Guzel T. Can round ligament of the liver patch decrease the rate and the grade of postoperative pancreatic fistula? *Pol Przegl Chir* 2016;**88**(2):63–67

11. Park JS, Lee DH, Jang JY, Han Y, Yoon DS. Use of TachoSil(®) patches to prevent pancreatic leaks after distal pancreatectomy: a prospective, multicenter, randomized controlled study. *J Hepatobiliary Pancreat Sci* 2016;**23**(2):110–117
12. Kato D, Sasaki T, Yamashita K, Shinya S, Nakashima R. Drain selection reduces pancreatic fistulae risk: a propensity-score matched study. *Hepatogastroenterology* 2015;**62**(138):485–492
13. Schoellhammer HF, Fong Y, Gagandeep S. Techniques for prevention of pancreatic leak after pancreatectomy. *Hepatobiliary Surg Nutr* 2014;**5**:276–287
14. Kawai M, Tani M, Yamaue H. Early removal of prophylactic drains reduces the risk of intraabdominal infections in patients with pancreatic head resection: prospective study for 104 consecutive patients. *Ann Surg* 2006;**244**(1):1–7
15. Bassi C, Molinari E, Malleo G. Early versus late drain removal after standard pancreatic resections: results of a prospective randomized trial. *Ann Surg* 2010;**252**:207–214
16. Adachi T, Kuroki T, Kitasato A, Hirabaru M, Matsushima H, Eguchi S. Safety and efficacy of early drain removal and triple-drug therapy to prevent pancreatic fistula after distal pancreatectomy. *Pancreatology* 2015;**15**(4):411–416
17. Kuroshima N, Tanaka T, Kuroki T, Adachi T, Ono S, Eguchi S. Triple-drug therapy to prevent pancreatic fistula after pancreatectomy in a rat model. *Pancreatology* 2016;**16**(5):917–921
18. Tanaka T, Kuroki T, Adachi T, Ono S, Eguchi S. Development of a novel rat model with pancreatic fistula and the prevention of this complication using tissue-engineered myoblast sheets. *J Gastroenterol* 2013;**48**(9):1081–1089
19. Machado MCC, Coelho AMM. Role of peritoneal macrophages on local and systemic inflammatory response in acute pancreatitis. *Intech Open* 2012;**8**:102–108
20. Caroline SM, Garry AA, Amanda JO. Association between canine pancreatic-specific lipase and histologic exocrine pancreatic inflammation in dogs: assessing specificity. *J Vet Diagn Invest* 2012;**24**(2):312–318.
21. Kitahata Y, Kawai M, Yamaue H. Clinical trials to reduce pancreatic surgery-review of randomized controlled trials. *Transl Gastroenterol Hepatol* 2016;**1**:4
22. Suc B, Msika S, Piccinini M. Octreotide in the prevention of intra-abdominal complications following elective pancreatic resection: a prospective, multicenter randomized controlled trial. *Arch Surg* 2004;**139**(3):288–294
23. Sarr MG; Pancreatic Surgery Group. The potent somatostatin analogue vapreotide does not decrease pancreas-specific complications after elective pancreatectomy: a prospective, multicenter, double-blinded, randomized, placebo-controlled trial. *J Am Coll Surg* 2003;**196**(4):556–564
24. Yeo CJ, Cameron JL, Lillemoe KD. Does prophylactic octreotide decrease the rates of pancreatic fistula and other complications after pancreaticoduodenectomy?: results of a prospective randomized placebo-controlled trial. *Ann Surg* 2000;**232**(3):419–429
25. Lowy AM, Lee JE, Pisters PW. Prospective, randomized trial of octreotide to prevent pancreatic fistula after pancreaticoduodenectomy for malignant disease. *Ann Surg* 1997;**226**(5):632–641
26. Fernandez CI, Jimenez CE, Taura P. Prospective randomized trial of the effect of octreotide on pancreatic juice output after pancreaticoduodenectomy in relation to histological diagnosis, duct size and leakage. *HPB* 2013;**15**(5):392–399
27. Caronna R, Diana L, Nofroni I, Sibio S. Effects of gabexate mesylate (FOY) on amylase and phospholipase A2 in human serum and pancreatic juice. *Dig Dis Sci* 2005;**50**(5):868–873
28. Yokoe M, Takada T, Mayumi T, Yoshida M, Isaji S, Wada K. Japanese guidelines for the management of acute pancreatitis: Japanese Guidelines 2015. *J Hepatobiliary Pancreat Sci* 2015;**22**(6):405–432
29. Villatoro E, Mulla M, Larvin M. Antibiotic therapy for prophylaxis against infection of pancreatic necrosis in acute pancreatitis. *Cochrane Database Syst Rev* 2010;**12**:5
30. Noor MT, Radhakrishna Y, Kochhar R, Singh K. Bacteriology of infection in severe acute pancreatitis. *J Pancreas* 2011;**12**(1):19–25
31. Leadros E, Antonakis PT, Albanopoulos K. Somatostatin versus octreotide in the treatment of patients with gastrointestinal and pancreatic fistulas. *Can J Gastroenterol* 2004;**8**(5):303–306
32. Prinz RA, Pickleman J, Hoffman JP. Treatment of pancreatic cutaneous fistulas with a somatostatin analog. *Am J Surg* 1988;**155**(1):36–42
33. Chan MM, Mengshol JA, Fish DN, Chan ED. Octreotide: a drug often used in the critical care setting but not well understood. *Chest* 2013;**144**(6):1937–1945
34. Allen PJ, Gönen M, Brennan MF. Pasireotide for postoperative pancreatic fistula. *N Engl J Med* 2014;**370**:2014–2022
35. Guotao L, Zhihui T, Yanbing D. Aspirin protects against acinar cells necrosis in severe pancreatitis in mice. *Biomed Res Int* 2016;**2016**:6089430