Proposal of chymotrypsin-based perioperative management in pancreatic surgery

Ishizawa T,1,2 Yamashita S,2 Mori K,2 Sakamoto Y,2 Hasegawa K,2 Kamiya M,3 Urano Y,4,5 Saiura A,1, and Kokudo N,2

1Cancer Institute Hospital, Japanese Foundation for Cancer Research
2Graduate School of Medicine (Hepato-Biliary-Pancreatic Surgery and Laboratory of Chemical Biology and Molecular Imaging) and Pharmaceutical Sciences (Laboratory of Chemistry and Biology), the University of Tokyo

Introduction

Postoperative pancreatic fistula (PF)
• The number of pancreatic resections is increasing > 40,000 / year in the US > 10,000 / year in Japan
• PF still occurs with an incidence of ~60% and remains the most serious complication of pancreatic surgery associated with mortality: 10% to 20% in high-volume hospitals

Problems in PF prevention
• No means of visualizing clear pancreatic juice leakage from pancreatic stump/anastomosis during surgery.
• In theory, pancreatic proteases that can directly cause tissue damages should be an optimal predictor for the development of clinically-relevant PF (CR-PF), rather than glycolytic enzymes like amylase.

Aims
1) To develop fluorescence imaging technique for intraoperative visualization of pancreatic juice leakage
2) To evaluate prognostic values of potential chymotrypsin activities in pancreatic juice for the development of CR-PF

Methods

1-1) Development of a “Chymotrypsin probe”
• We developed a “Chymotrypsin probe (glutathione-phenylethylamine hydroxymethyl rhodamine green [gPhe-HMRG] with added trypsin)” that emits light when hydrolyzed with chymotrypsin derived from pancreatic chymotrypsinogen.
• Efficacy of this probe was evaluated using ex vivo samples of obtained from 32 patients undergoing pancreaticoduodenectomy (PD).
• Fluorescence imaging using the Chymotrypsin probe was specific to pancreatic juice.

1-2) Simulation of in vivo fluorescence imaging using the Chymotrypsin probe
• In swine distal pancreatectomy models, efficacy of the fluorescence imaging of pancreas juice was evaluated, by spraying the Chymotrypsin probe directly onto the pancreatic stump divided and closed with a stapler device.
• Fluorescence signals were observed by naked-eye examinations through yellow glass filter or with a newly-devised laparoscopic imaging system.

1-3) Development of a “Chymotrypsin probe”
• The chymotrypsin probe was sprayed onto the filter papers that had been attached to the patients’ pancreatic stump during resection.
• Fluorescence patterns could be classified into the Duct, Diffuse, and Negative types.

1-4) Prognostic values of potential chymotrypsin activity in pancreatic juice for development of CR-PF
• In 81 patients undergoing PD with pancreaticojunostomy, pancreatic juice samples were obtained from totally-external drainage tube inserted into the main pancreatic duct.
• Postoperatively, potential chymotrypsin activities in pancreatic juice (P-Chy) was measured with the Chymotrypsin probe, after converting chymotrypsinogen to chymotrypsin by added trypsin.
• Amylase levels in pancreatic juice (P-Amy) and those in drained abdominal fluids (D-Amy) were also measured.
• Predictive values of P-Chy, P-Amy, and D-Amy for development of CR-PF were evaluated.

Results

Duct type PO (n=1)
• The chymotrypsin probe was sprayed onto the filter papers that had been attached to the patients’ pancreatic stump during resection.
• Fluorescence patterns could be classified into the Duct, Diffuse, and Negative types.

Diffuse type PO (n=7)
CR-PF (ISGPF grade B or higher)
• CR-PF developed in 19 patients (23%).
• ROC analyses revealed that P-Chy on the first postoperative day (POD) was associated with the highest ability in prediction of CR-PF, among other predictive factors based on amylase levels.

Negative type PO (n=7)

• CR-PF developed in 19 patients (23%).
• ROC analyses revealed that P-Chy on the first postoperative day (POD) was associated with the highest ability in prediction of CR-PF, among other predictive factors based on amylase levels.

Conclusions

• Fluorescence imaging using the Chymotrypsin probe allows real-time visualization of pancreatic leak.
• If the Chymotrypsin probe can be sprayed directly to the pancreatic stump in the patient’s abdominal cavity, the present technique may enable pinpoint closure of pancreatic leak and selective use of prophylactic abdominal drains.
• Intra- and postoperative evaluation of potential chymotrypsin activities in pancreatic juice may predict development of CR-PF more accurately than the current amylase-based diagnostic criteria, because it is not amylase, a glycolytic enzyme, but proteases that can cause severe tissue damages due to PF.

Correspondence:
Takeaki Ishizawa, MD, PhD, FACS
E-mail: tak-ky@umin.ac.jp

Effect of circadian rhythm and postoperative recovery time on pancreatic enzyme excretions

• Amylase excretion was more susceptible to circadian rhythm and postoperative elapsed time than chymotrypsinogen excretion estimated as chymotrypsin activity.