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Cystic Duct Leak Closure Using Poly-N-acetyl Glucosamine : Results in a Swine Model

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ABSTRACT

Poly-N-acetyl glucosamine (p-Glc-NAc) is a biodegradable and biocompatible gel derived from a marine diatom which solidifies at basic pH. We examined the safety and efficacy of endoscopic occlusion of cystic duct leaks with p-Glc-NAc in Yucatan pigs. In two pigs, p-Glc-NAc was injected into the intrahepatic ducts to determine its effect on normal bile ducts. In three pigs, laparoscopic cholecystectomy with a patent cystic duct was performed without subsequent injection of p-Glc-NAc to determine the reliability of the experimental model. Eight pigs received injections into the cystic duct stump of 2.5% p-Glc-NAc mixed with contrast agent by means of an endoscopic retrograde cholangiopancreatography catheter immediately after laparoscopic cholecystectomy with a patent cystic duct. After 1, 7, 21, or 90 days the pigs underwent endoscopic retrograde cholangiography and were then killed. Autopsy showed that the p-Glc-NAc had been delivered properly and had occluded the cystic duct stump in 6 of the 8 pigs. However, the p-Glc-NAc was not delivered properly because of breakdown of the injection system in 1 pig and a technical failure leading to misinjection in 1 pig. In both these pigs peritonitis developed, the cystic duct remained patent, and no p-Glc-NAc was found in the bile duct. Our results suggest that endoscopic occlusion of cystic duct leaks with p-Glc-NAc is safe and effective. Use of this material would eliminate the need for sphincterotomy or stenting, decrease costs, and decrease the risk of complications. (Jikeikai Med J 2005 ; 52 : 31-8)

Key words : biliary leakage, poly-N-acetyl glucosamine, laparoscopic cholecystectomy, endoscopic therapy

INTRODUCTION

Bile leak is an uncommon but serious complication of laparoscopic cholecystectomy. If bile leak occurs, endoscopic treatment is usually performed first. If this treatment is unsuccessful, other treatments, including therapeutic drainage and surgical repair by means of laparoscopy or laparotomy, can be attempted[1].

Poly-N-acetyl glucosamine (p-Glc-NAc ; Marine Polymer Technologies, Inc., Danvers, MA, USA) is a polysaccharide polymer isolated from controlled aseptic cultures of a marine microalga[2]. Isolated p-Glc-NAc is of high purity and consistency with an average molecular weight of 2×10^6 Dalton and can be formulated as a sterile, fully biocompatible gel[3]. The polymer has been tested in accordance with Food and Drug Administration biocompatibility guidelines, including sensitization assays and tests for skin irritation, systemic toxicity, cytotoxicity, mutagenicity, subchronic toxicity, and pyrogenicity. This gel begins to precipitate at a pH of 7 or greater,

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a condition present in the bile duct. External application of p-Glc-NAc gel to bleeding wounds results in rapid hemostasis through stimulation of erythrocyte aggregation. When injected into the ear veins of rabbits, p-Glc-NAc gel immediately occludes the vessels and recruits inflammatory cells, eventually leading to complete replacement of the veins by connective tissue[4]. We have used this material to treat esophageal varices in a swine model and have obtained good results[5].

These findings suggest that the p-Glc-NAc gel can be used to induce plug formation in the cystic duct. We tested this hypothesis in a swine model with laparoscopically created cystic duct leaks.

MATERIALS AND METHODS

p-Glc-NAc

For endoscopic injection, an initial p-Glc-NAc formulation (F1) was prepared as a 2.5% sterile gel. In all cases, the p-Glc-NAc lactate salt was dissolved in the radiographic contrast agent iohexol (Omnipaque 300, Sanofi Winthrop Pharmaceuticals, New York, NY, USA). This F1 gel formulation was chosen so that the material could be traced over time in subject animals by means of fluoroscopy and histologic examination. Another reason the F1 gel formulation was chosen was because it recruits inflammatory cells, thereby stimulating the development of connective tissue[4].

The viscous F1 gel was loaded into a 10-mL syringe with high-pressure capability (LeVeen Inflator, Microvasive, Boston Scientific, Natick, MA, USA) and injected through the guide wire port of a triple-lumen endoscopic retrograde cholangiopancreatography (ERCP) catheter (Microvasive).

Animals

The study protocol was approved by the institutional review board of the Medical University of South Carolina. Thirteen male Yucatan pigs weighing 20 to 25 kg were used for this study (Table 1). All endoscopic procedures were performed, after endotracheal intubation and under general anesthesia, by the same endoscopists (K.M. and R.H.).

Table 1. Characterization of pigs and results of endoscopic occlusion of p-Glc-NAc F1-gel injections

Pig	1	2	3	4	5	6	7	8	9	10	11	12	13
	Intrahepatic Control		LCDP Control*			Acute model (1 day later)		Subacute model (1 week later)		Mid-term model (3 weeks later)		Long-term model (3 months later)	
Injected site	left intrahepatic biliary duct		None			Cystic duct stumps							
Gel volume (mL)	2.0	2.1				2.0	1.9	2.1	2.0	n/a	2.0	2.0	2.0
Leak on ERC on 7 days								−	+	+	−	−	− (Fig. 3)
Leak on ERC on 21 days											−	−	− (Fig. 4a)
Leak on ERC on 90 days												−	− (Fig. 4b)
Cystic duct	patent	patent	patent	patent	patent	occlusive (Fig. 5a)	not occlusive	occlusive (Fig. 5b)	patent	patent	occlusive (Fig. 5c)	occlusive (Fig. 5d)	occlusive
p-Glc-NAc location	gall-bladder	left hepatic duct	none	none	none	cystic duct	cystic duct	cystic duct bifurcation		none	cystic duct	none	none
Peritonitis	none	none	mild acute	mild acute	minimal acute	none	mild	none	moderate	severe	mild chronic	none	none
Liver	Normal												

*LDCP: laparoscpic cholecystectomy, cystic duct patient

Preliminary study, part 1

Endoscopic occlusion of the left intrahepatic biliary tract

The aim of this part of the study was to evaluate the system for injecting p-Glc-NAc through an ERCP catheter into the biliary duct and the possibility of using p-Glc-NAc to seal the biliary tract without bypass bile juice flow, different from the common bile duct with cystic duct stump, which can act as an alternative passage for bile juice. Pigs 1 and 2 were used for the left intrahepatic biliary tract injection of p-Glc-NAc. One day later, the pigs were killed and examined at autopsy, and the efficacy of p-Glc-NAc for occluding the biliary tract was evaluated histologically.

Preliminary study, part 2

Laparoscopic cholecystectomy and creation of biliary leaks

Pigs 3 to 13 underwent laparoscopic surgery. The cystic duct was ligated with two clips, then cut proximally to the clip. One clip was left as a marker of the cystic duct stump without ligating the cystic duct. To evaluate the open stump histologically, pig 3 and pigs 4 and 5 were killed and examined at autopsy 6 hours later and 1 day later, respectively.

Preliminary study, part 3

Endoscopic occlusion of biliary leaks

Endoscopic injection of p-Glc-NAc to the cystic duct stump was performed for pigs 6 to 13, after inserting the guide wire with the triple-lumen ERCP catheter (Microvasive) into the resected cystic duct with a diagnostic duodenoscope (JF-100, Olympus America, Inc., Melville, NY, USA). Once the tip of the cannula was placed in the cystic duct, the guide wire was pulled out. Iohexol and 2.5% p-Glc-NAc were injected through the guide wire port of the catheter under fluoroscopic guidance. Once the injected material had filled the cystic duct stump, the cannula was gently pulled out.

Follow-up

Pigs 8 to 13 were followed up with ERCP on days 7, 21, and 90 to verify cystic duct occlusion and to rule out biliary leaks by injecting iohexol. At autopsy, the cystic duct stump was observed *in situ* for evidence of leaks or any damage to the surrounding organs. The specimen was fixed in formalin, and cross-sectional slices were obtained and stained with hematoxylin and eosin for light microscopy. The state of the cystic duct (patent or occluded) and the presence of gel or periductal inflammation were examined.

The development of an antibody response to the gel was determined with an indirect enzyme-linked immunosorbent assay[6]. Sera from mice immunized against the F1 gel formulation served as positive controls. Sera from pigs 3, 4, and 5 were used as negative controls. The pigs were tested 1 day (pigs 6 and 7), 7 days (pigs 8 and 9), 21 days (pigs 10 and 11), and 90 days (pigs 12 and 13) after p-Glc-NAc injection.

Results

Left intrahepatic biliary duct injection

p-Glc-NAc injected into the right intrahepatic biliary duct in pigs 1 and 2 was visualized fluoroscopically. One day later, the pigs were examined at autopsy. Histologic examination showed that the lumen of the left intrahepatic biliary duct was not occluded with p-Glc-NAc F1 gel. The p-Glc-NAc F1 gel was seen in the gallbladder of pig 1 and in the left intrahepatic duct, with no associated inflammatory response, of pig 2.

Cystic duct leak model

Pig 3 was examined at autopsy 6 hours after laparoscopic surgery. A bile juice leak was observed. Histologic examination confirmed the patency of the cystic duct. Pigs 4 and 5 were examined at autopsy 1 day after laparoscopic surgery. Bile juice leaks were observed. Histologic examination showed partial patency of the cystic duct and accumulation of

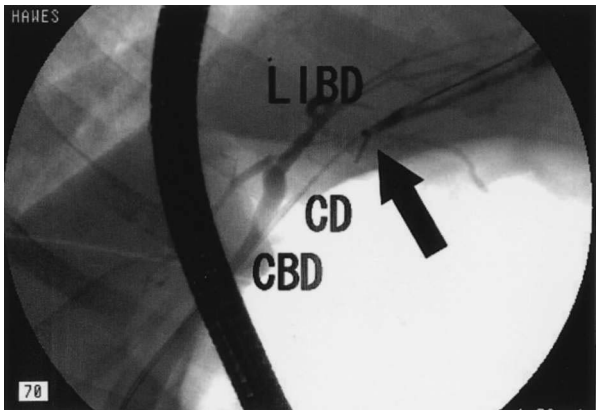

Fig. 1. ERC image before p-Glc-NAc injection. The arrow indicates the cystic duct stump. Iohexol and the guide wire can be seen protruding from the cystic duct stump. A clip is visible near the cystic duct stump.
CBD : common bile duct CD : cystic duct
LIBD : left intrahepatic biliary duct

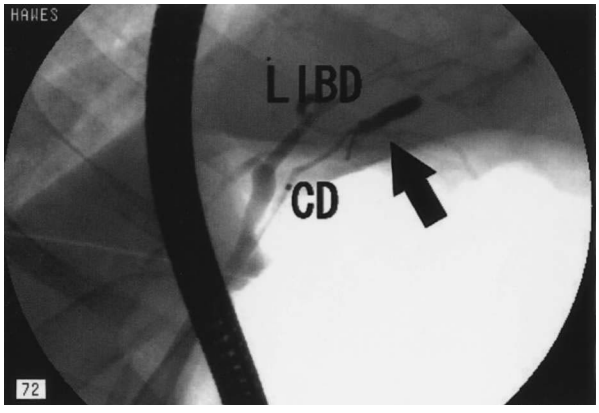

Fig. 2. p-Glc-NAc is sealing the stump. The arrow indicates the p-Glc-NAc with iohexol.
CD : cystic duct LIBD : left intrahepatic biliary duct

inflammatory cells. In pigs 6 to 13, cystic duct leaks were visualized fluoroscopically after iohexol injection (Fig. 1).

Endoscopic injection of p-Glc-NAc

After the cystic duct leak was confirmed fluoroscopically, p-Glc-NAc and iohexol were injected into the cystic duct stumps (Fig. 2). The mean total gel volume was 2.0 mL (range, 1.9 to 2.1 mL). The cannula was withdrawn 10 seconds after complete occlusion of the cystic duct stump with p-Glc-NAc was

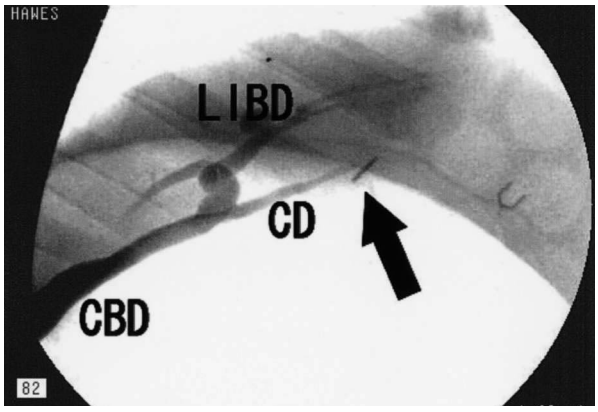

Fig. 3. ERC image 1 week after injection in pig 13. The arrow indicates the cystic duct stump. No leak was visualized.
CBD : common bile duct CD : cystic duct
LIBD : left intrahepatic biliary duct

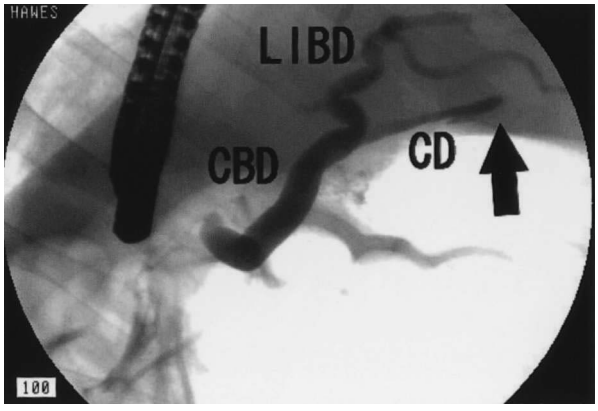

Fig. 4a. ERC image 3 weeks after injection in pig 13. The arrow indicates the cystic duct stump. No leak was visualized.

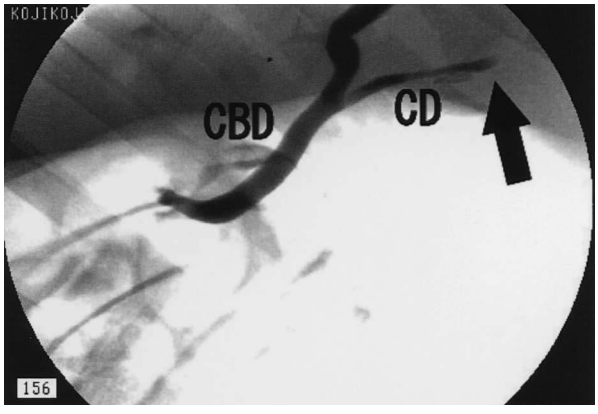

Fig. 4b. ERC image 3 months after injection in pig 13. The arrow indicates the cystic duct stump. No leak was visualized.
CBD : common bile duct CD : cystic duct
LIBD : left intrahepatic biliary duct

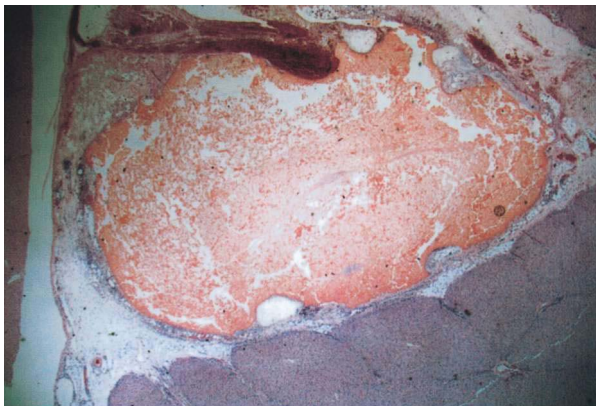

Fig. 5a. Microscopic image of cystic duct stump in pig 6. The cystic duct is partially occluded by p-Glc-NAc with localized neutrophilic reaction.

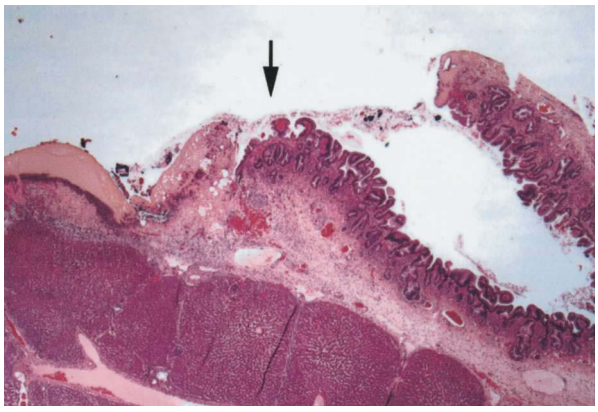

Fig. 5c. Microscopic image of cystic duct in pig 11. The cystic duct is completely occluded. The arrow indicates the cystic duct stump.

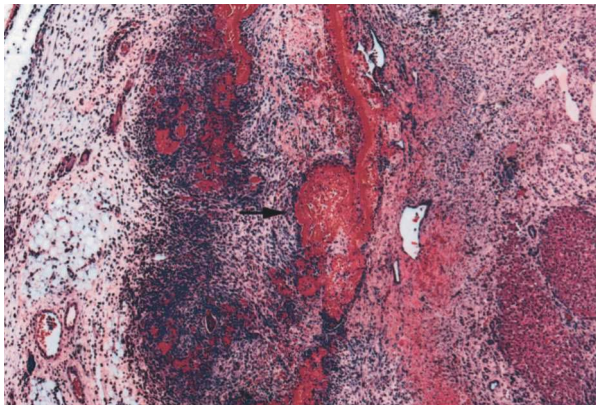

Fig. 5b. Microscopic image of cystic duct stump in pig 8. The cystic duct is completely occluded by p-Glc-NAc. The arrow indicates the epithelium of the bile duct.

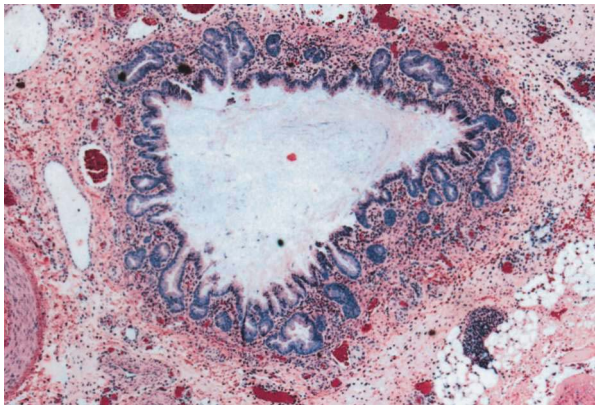

Fig. 5d. Microscopic image of cystic duct in pig 12. The p-Glc-NAc is not seen in the cystic duct.

visualized.

Fluoroscopic follow-up

Pigs 8 to 13 were followed up by injecting iohexol on days 7, 21, and 90. The injected iohexol was not visualized on day 7. Complete occlusion was confirmed by injecting iohexol in all cases (Fig. 3, 4a, b). In 6 of 8 cases (75%), p-Glc-NAc was delivered properly ; in each of these 6 cases, p-Glc-NAc sealed the cystic duct effectively.

The p-Glc-NAc was not delivered properly in two cases : once because of breakage of the injection system which prevented delivery of p-Glc-NAc and another time because of a technical failure that caused p-Glc-NAc to be injected into the peritoneum rather than into the cystic duct stump. Peritonitis developed in both cases (moderate in 1 case, severe in 1 case), and the cystic ducts remained patent. No p-Glc-NAc was subsequently found in the bile duct, and no abnormalities were seen in the biliary tree.

Autopsy

The cystic duct stumps were visible macroscopically. On histologic examination on day 1 the F-1 gel precipitations appeared as homogenous pinkish areas with hematoxylin and eosin staining. The cystic duct was partially occluded by p-Glc-NAc and showed a localized neutrophilic reaction (Fig. 5a). Seven days (Fig. 5b) and 21 days (Fig. 5c) after injec-

tion, the cystic duct was completely occluded by p-Glc-NAc. Ninety days after injection, p-Glc-NAc was not seen in the cystic duct (Fig. 5d). In pigs 9 and 10, macroscopic evidence of bile peritonitis was observed.

Enzyme-linked immunosorbent assay

No antibodies against F1 gel were detected 7, 21, or 90 days after gel injection in any of the tested pigs (pigs 6 to 13) at any ratio of serum dilution.

DISCUSSION

Laparoscopic cholecystectomy is the current treatment of choice for cholelithiasis. Although cystic duct leaks are a rare complication of open cholecystectomy, they are more common after laparoscopic cholecystectomy[7]. Kozarek et al have reported that 5 of 29 cases of ERCP-confirmed bile duct injury during laparoscopic cholecystectomy were injuries of the cystic duct, although their data were obtained soon after laparoscopic cholecystectomy was introduced. Some leaks remain asymptomatic and close spontaneously without further treatment. However, persistent leakage may result in biloma, abscess, peritonitis, or biliocutaneous fistula. Excessive bile loss can lead to metabolic abnormalities and hypovolemia[8].

Persistent leaks have historically required surgery, although percutaneous transhepatic drainage now offers a less invasive alternative. However, endoscopic methods have now replaced surgical or percutaneous intervention in most cases. The goal of endoscopic treatment is to decrease the pressure gradient between the bile duct and the duodenum and encourage bile to flow into the intestine rather than through the leak[9].

Current treatments for cystic duct leaks include sphincterotomy, nasobiliary drainage, and transpapillary stenting. Postoperative sphincterotomy is the most commonly reported treatment, with several series claiming higher healing rates with sphincterotomy than with biliary stenting[10,11]. When used for patients without risk factors, such as stricture, stenting is as effective as sphincterotomy[12]. However, 5% of cystic duct leaks do not heal well after stenting[13]. Two cases of cystic duct leak, despite preoperative sphincterotomy, after laparoscopic cholecystectomy have been reported[13]. These cases suggest the importance of directly treating the cystic duct leaks rather than changing the pressure gradient.

Current treatments have several disadvantages. Sphincterotomy can cause permanent sphincter damage, which can increase the risk of infections. The procedure itself carries a small but definite risk of bleeding. Stenting requires that the patient undergo ERCP a second time to remove the stent. Nasobiliary drainage is extremely uncomfortable for any length of time.

However, our treatment with p-Glc-NAc has several advantages. Sphincterotomy is not required before stent placement, and stent placement might be avoided entirely.

p-Glc-NAc is a recently characterized polysaccharide polymer. It has been shown to be a suitable delivery system for sustained release of cytokines and peptides at local sites and has been shown to provide effective hemostasis by stimulating erythrocyte aggregation. Consequently, p-Glc-NAc acts independently from platelet function and conventional plasma coagulation, both of which may be impaired in patients with varices caused by liver cirrhosis. p-Glc-NAc is equally effective in healthy animals and in animals with induced or inherited coagulopathies.

Compared with other adhesive agents, such as cyanoacrylates, p-Glc-NAc F-1 gel has several advantages. First, it is fully biodegradable and biocompatible. Therefore, p-Glc-NAc F-1 gel is fundamentally nonantigenic and is absorbed into the human body naturally. Second, it can be dissolved with contrast agents, such as inohexol, whereas other adhesives cannot be dissolved easily with contrast agents. Third, the viscosity of p-Glc-NAc F-1 gel can be adjusted as required. We used a concentration of 2.5% in this study, although a thinner gel can be prepared if necessary.

In this study, the feasibility of using p-Glc-NAc to plug leaks of the cystic duct stump was confirmed in both the acute phase and long term. We found that

p-Glc-NAc gel was replaced by connective tissue within 90 days, a result similar to that of our previous study in rabbit ear veins[4]. We also found no antibodies against p-Glc-NAc in the sera of pigs. This finding suggests that p-Glc-NAc would be safely replaced by connective tissue after injection.

The p-Glc-NAc was delivered properly in 6 of 8 cases (75%) in our study. In each of these 6 cases, the cystic duct was sealed effectively. We believe that our method is effective and produces no complications if p-Glc-NAc is injected properly.

Peritonitis developed because of bile leakage in two cases ; one case was caused by a malfunction of the delivery system which prevented effective delivery of p-Glc-NAc, and the other was caused by a technical failure in which p-Glc-NAc was injected into the peritoneum rather than into the cystic duct stump. These results suggest that the cystic duct leakage can cause bile peritonitis, which must first be treated endoscopically. Because of possible malfunction of the delivery system, we intend to design a more stable system made from metal rather than plastic. To minimize the chance of technical failure, we recommend the cannula be withdrawn 1 minute rather than 10 seconds after occlusion of the duct stump has been visualized so that the p-Glc-NAc can solidify more firmly.

This experiment was a pilot study to evaluate the feasibility of using p-Glc-NAc to seal fistulas and leaks in the pancreatobiliary tree. Further experiments could be designed to assess the use of this material at other sites, such as pancreatic leaks. Endoscopic occlusion of cystic duct leaks with p-Glc-NAc appears to be safe and effective. Use of p-Glc-NAc would obviate sphincterotomy or stenting, decrease costs, and lower the risk of complications. Further studies would be necessary to enhance the safety and efficacy of this method using the constant delivering system of this material through the ERCP catheter.

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Disclosure Statement

John N. Vournakis, PhD, is a participant in Marine Polymer Technologies, Inc.

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