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Novel flower-type covered metal stent to prevent cholecystitis: experimental study in a pig model

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Abstract
Background Covered self-expandable metal stent (CSEMS) has the risk of obstruction of the cystic duct, and the main and branch pancreatic ducts due to strong radial force and covering material, which results in cholecystitis and pancreatitis. A flower-type covered self-expandable metal stent (F-CSEMS) having a five-petal-shaped design with side grooves was constructed to prevent the obstruction of the cystic duct orifice. This study investigated the value of the F-CSEMS in protection for cholecystitis in a pig model.
Methods Fourteen pigs randomly underwent endoscopic placement of either F-CSEMS or conventional CSEMS (C-CSEMS). The stent was placed across the cystic duct orifice to impede bile drainage from the gallbladder. Drainage was checked at 24, 48, 120 and 168 h after implantation. Blood was collected at baseline, on days 2 and 7 following implantation. The animals were killed for histologic evaluation on day 7.
Results All stents were successfully inserted into bile duct without any procedure-related complications. At 48 h, the rate of contrast drainage from the gallbladder was higher in the F-CSEMS group than the C-CSEMS group without significant difference (71.4 vs. 28.6 % $p = 0.28$). C-CSEMS was associated with higher levels of C-reactive protein (35.2 vs. 20.5 μg/dl, $p = 0.03$) and histologic inflammatory scores of gallbladder (score 4 vs. 2; $p = 0.03$).
Conclusion The F-CSEMS appears safe and helpful to prevent cholecystitis without disturbance of bile flow in a pig model.

Keywords Bile duct stent · Self-expanding metal stent (SEMS) · Flower-type covered metal stent · Cholecystitis · Pig model

The covered self-expandable metal stent (CSEMS) has been widely used for the palliation of malignant biliary obstruction. The advantages of CSEMS are prevention of tumor ingrowth, and ease of removal in addition to a large lumen and long patency of uncovered stents. CSEMS use has also increased in benign biliary and pancreatic strictures as an alternative to multiple plastic stents, due to reduced necessity of endoscopic procedures and good long-term outcome [1].

However, CSEMS has the risk of obstruction of the cystic duct, and the main and branch pancreatic ducts due to strong radial force and covering material, which results in cholecystitis and pancreatitis. The incidence of cholecystitis with the use of CSEMS ranges from 5.8 to 11.5 % in distal biliary malignancy [2, 3]. Although no pancreatic sepsis has been reported, the rate of acute pancreatitis ranging from 0 to 9.3 % in benign pancreatic duct strictures has been described [4, 5].

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Cholecystitis after biliary stenting could occur because of bacterial colonization of bile after endoscopic procedures or impairment of gallbladder (GB) function caused by obstruction of the cystic duct with tumor involvement, which could result in filling of the GB with nonsterile contrast medium. Early cholecystitis that develops within days of stent insertion can be associated with the occlusion of the cystic duct by radial force or the stent covering material [6].

Recently, we developed a flower-type CSEMS (F-CSEMS) to prevent the occlusion of cystic duct and impaired GB emptying. The aim of this study was to investigate the technical feasibility and safety of the novel stent and to confirm whether this novel CSEMS could prevent acute cholecystitis compared with the conventional CSEMS (C-CSEMS) in a pig model.

Materials and methods

This study was reviewed and approved by the institute's Institutional Animal Care and Use Committee at the Laboratory Animal Research Center of Samsung Biomedical Research Institute.

Preparation of F-CSEMS and C-CSEMS

The novel F-CSEMS and commercial C-CSEMS (S&G Biotech, Seongnam, Gyunggi, Korea) were 8 mm wide and 80 mm long when fully expanded and mounted on an 8-F stent introducer set. Expanded polytetrafluoroethylene (e-PTFE) was selected as the covering material of both stents. The F-CSEMS is a five-petal-shaped stent with grooves at the sides parallel to the long axis. It is specifically designed to prevent the occlusion of cystic duct and to maintain GB emptying (Fig. 1).

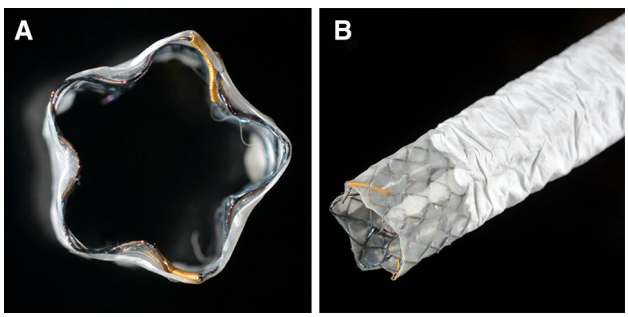


Fig. 1 Flower-type covered self-expanding metal stent (CSEMS). **A** Cross-sectional photograph demonstrating the pentagonal shape with five petals. **B** Longitudinal image illustrating the five side grooves oriented parallel to the long axis

Animal model

A total of 14 female domesticated pigs (average weight 30 kg) were randomly divided into two groups ($n = 7$ per group). Endoscopic retrograde cholangiopancreatography (ERCP) with a duodenoscope (TJF240; Olympus, Tokyo, Japan) was performed with a standard technique using papillotome catheters and guide wires under the fluoroscopy guidance. Baseline cholangiograms were obtained. After the cannulation of the cystic duct in each pig, 25 ml of contrast dye and 5 ml of their own gastric contents were injected to fill the GB. The F-CSEMS or C-CSEMS was placed across the opening of the cystic ducts to impede bile drainage from the GB.

Follow-up and histologic examination

After endoscopic stent placement, all animals were maintained on their usual diet for 7 days, at which time the animals were euthanized with an overdose of intravenous potassium chloride. The fluoroscopic images were performed to check the drainage of contrast from the GB at 24, 48, 120 and 168 h. Blood was collected at baseline, on days 2 and 7 after stent placement. During necropsy, all surrounding tissues were also examined for signs of infection, peritonitis and necrosis. The liver, GB, common bile duct and duodenum were excised en bloc. GB and bile ducts were incised longitudinally, and the mucosa was inspected. The sectioned tissue samples were stained with hematoxylin and eosin and inspected by a pathologist experienced in gastrointestinal diseases (K.T.J.). Histopathologic examination was performed in a blinded manner using a modification of the previous histologic scoring system to determine the extent of inflammation (1 = minimal, 2 = confined to mucosa, 3 = extends to muscle), severity of neutrophil infiltration (0 = absent, 1 = mild, 2 = moderate, 3 = severe) and presence of mucosal ulceration (0 = absent, 2 = focal, 4 = diffuse) [7, 8].

Statistical analysis

This study was designed as a pilot experimental study using the minimum amount of animals required for testing and comparing a novel instrument. Fisher's exact test for categorical data and the unpaired *t* test for continuous data were used to compare the two groups. The Mann–Whitney test was used to assess the significance of between-group histopathologic findings. All tests were two-sided, and the significance level was set at 5 %. Statistical analyses were performed using STATA 12.0 (Stata, College Station, TX, USA).

Results

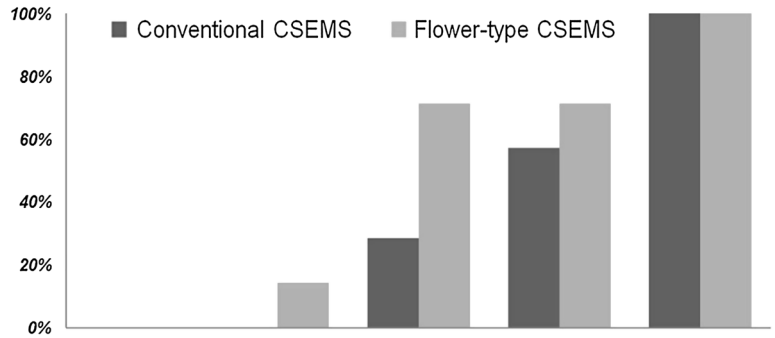
All 14 pigs had successful deployment of stent across the opening of cystic duct with no procedure-related complications. For the next 7 days, monitoring of the pigs included clinical parameters and daily food intake. All pigs survived this period. Early migration (within 7 days) occurred in one pig of the C-CEMS group and two pigs of the F-CSEMS group. The contrast emptying from the GB was assessed at specified times. As time passed, the rate of contrast emptying increased. At 168 h, no remaining GB contrast was observed in any of the pigs. The difference in the rate of contrast emptying was greatest between the F-CSEMS and C-CSEMS groups at 48 h (71.4 vs. 28.6 %; $p = 0.28$; Fig. 2). No significant differences of laboratory finding except C-reactive protein (CRP) levels were seen between both groups. CRP levels were significantly different on day 2 (35.2 vs. 20.5 μg/dl, $p = 0.03$; Fig. 3). No sign of infection and peritonitis were observed in all surrounding tissue including liver, duodenum, common bile duct and GB during laparotomy. Three pigs of the C-CSEMS group displayed a distended GB with the presence of foul-smelling greenish material. Histologic examination of GB tissue revealed the mild-to-moderate inflammatory changes, while no specific finding was observed in the common bile duct. In comparison with histologic inflammatory scores, the C-CSEMS group had a higher score than the F-CSEMS group. The extent of inflammation and total score demonstrated a statistically significant difference ($p = 0.04$ and $p = 0.03$, respectively; Table 1).

Discussion

A fully covered metal stent is widely used for malignant and benign biliopancreatic strictures in terms of prevention of tissue ingrowth and removability. On the other hand, placement of fully covered metal stents may generate risk of obstruction of the orifice of the cystic duct or the side branch of the pancreatic duct due to covering of the membrane, resulting in cholecystitis or pancreatitis. The novel covered metal stent with side grooves was developed to prevent the impaired flow of bile fluid and pancreatic juice. This is the first animal study evaluating the safety and efficacy of placement of the F-CSEMS in the bile duct. This novel CSEMS has earlier emptying of contrast than C-CSEMS and protects against development of acute cholecystitis judged by CRP level and histologic inflammatory score.

To reproduce acute cholecystitis after the placement of CSEMS, an animal model involving CSEMS placement across the opening of the cystic duct to impede bile drainage from the GB was employed. Because of the limited number of pigs and low incidence of acute cholecystitis with use of CSEMS, an additional step was needed to heighten vulnerability to acute cholecystitis. Instead of injection of bacterial suspension, injection of the animals' own gastric contents was performed as an additional step. Our modified method is thought to be adequate for evaluating the prevention of CSEMS-related cholecystitis because manipulation of the biliary tree during ERCP (such as injection of nonsterile contrast material) in patients with malignant biliary obstruction could introduce nonsterile bile and/or contrast material into the GB [9].

Theoretically, uncovered stents with their open interstices can allow sufficient GB drainage to avoid cholecystitis. Although cholecystitis after stent insertion is rare for uncovered metal stents compared with covered metal stents [10, 11], cholecystitis could develop after placement of uncovered stents. This phenomenon is related to the fact that the cystic duct invasion of tumors is a major risk factor for acute cholecystitis after SEMS placement [6]. If the orifice of cystic duct is involved by tumor, bile flow is not obstructed completely, but pronounced impedance of bile



	0 hr	24 hr	48 hr	120 hr	168 hr
Conventional CSEMS	0 (0%)	0 (0%)	2 (28.5%)	4 (57.1%)	7 (100%)
Flower-type CSEMS	0 (0%)	1 (14.2%)	5 (71.4%)	5 (71.4%)	7 (100%)
p-value	1.00	1.00	0.28	1.00	1.00

Fig. 2 Comparison of contrast emptying rate from the gallbladder between conventional covered self-expanding metal stent (CSEMS) and flower-type covered self-expanding metal stent (CSEMS)

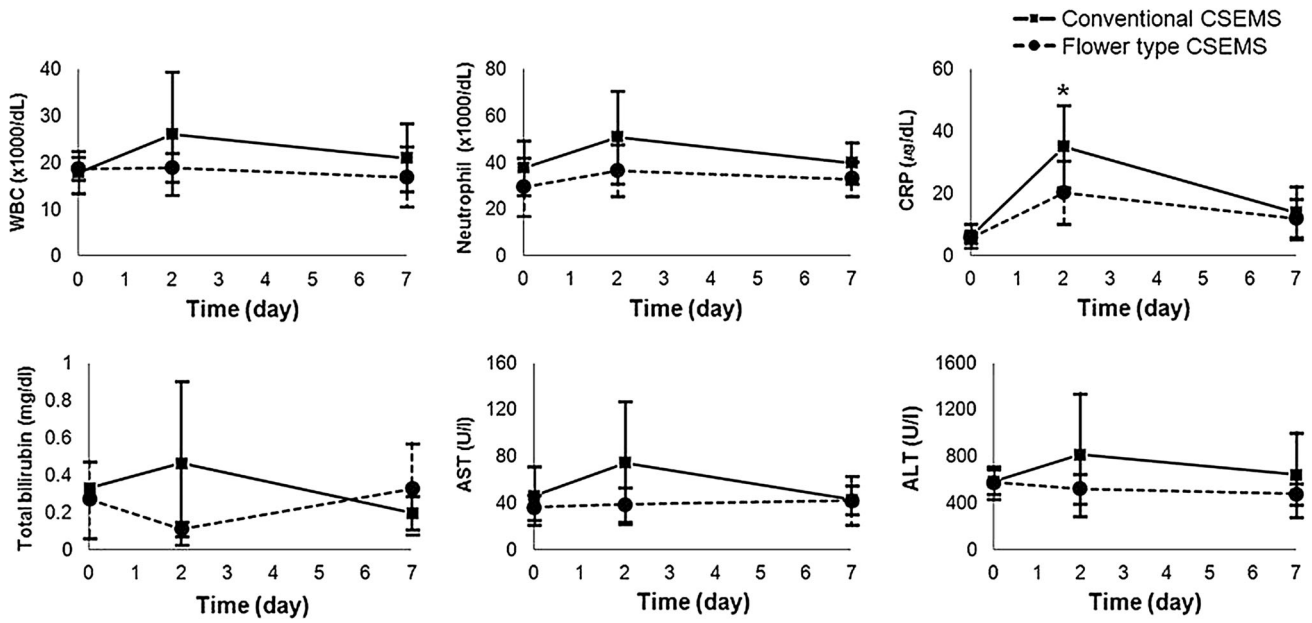


Fig. 3 Comparison of laboratory finding between conventional covered self-expanding metal stent (CSEMS) and flower-type covered self-expanding metal stent (CSEMS). *CRP levels are significantly different on day 2 (35.2 vs. 20.5 μg/dl; $p = 0.03$)

Table 1 Comparison of gallbladder inflammation using histologic score system between conventional covered self-expanding metal stent (CSEMS) and flower-type covered self-expanding metal stent (CSEMS)

	Conventional CSEMS	Flower-type CSEMS	*P* value[†]
Extent of inflammation, median (range)	2 (1–2)	1 (1–2)	0.044
Severity of neutrophil infiltration, median (range)	2 (1–2)	1 (0–2)	0.068
Presence of mucosal ulceration, median (range)	0 (0–4)	0 (0–0)	0.173
Total score, median (range)	4 (2–8)	2 (1–4)	0.032

[†] Histologic scores are compared using the Wilcoxon's rank-sum nonparametric test

flow may be created. A light compression by uncovered stent expansion could lead to impaired efflux of GB bile and development of cholecystitis. Presently, the F-CSEMS could prevent impairment of bile drainage and development of acute cholecystitis. The advantages of F-CSEMS to conventional metal stent are as follows. First, the direct compression of the orifice can be avoided because of the minimal contact surface resulting from the cross-sectional pentagonal shape. Second, F-CSEMS could channel bile flow because of the five longitudinal grooves. The novel stent design may lessen impairment of bile drainage and development of acute cholecystitis under even the involvement of cystic duct orifice.

CSEMS placement in the pancreatic duct may increase the risk of pancreatitis, since the covering material can obstruct the side branches. Although a previous study reported no severe complications related to side branch blockage [5], this result could be explained as a "forgiving" effect of end-stage pancreatitis with exhausted exocrine function. Therefore, complications related to side branch blockage can occur after placement of CSEMS in mild-to-moderate chronic pancreatitis with sufficient exocrine function. F-CSEMS may be helpful to prevent this complications related to CSEMS, even in mild-to-moderate chronic pancreatitis, because the longitudinal grooves are expected to channel pancreatic juice. However, we planned to place a stent only in the bile duct because it is practically difficult to place a metal stent in the small normal porcine pancreatic duct. Further animal studies or human studies are needed to validate the safety and efficacy of F-CSEMS for pancreatic duct stricture in chronic pancreatitis.

Several studies have reported stent migration in 6 and 12 % of the covered SEMS [12, 13]. The present rate was higher (14.2 % in the C-CSEMS group and 28.5 % in the F-CSEMS group). This may reflect the use of pigs with normal bile ducts. Therefore, the flower-type CSEMS is

expected to have lower stent migration rate in patients with stricture. Another factor accounting for the stent migration is that our stent was a prototype that lacked an anti-migration system, such as flared distal ends. Because no stent migration was observed in covered SEMSs with flared ends in a recent study [14], the migration rate could be expected to be reduced if the F-CSEMS incorporated an anti-migration system.

This animal study presents several limitations. First, the number of animals was relatively small due to the pilot study design. Second, the animals were observed only for 1 week after the placement of CSEMS. Long-term data, such as stent patency and stent dysfunction, were not collected. However, 7 days was long enough to confirm whether F-CSEMS could prevent acute cholecystitis.

In conclusion, the new flower-type CSEMS is technically feasible and safe for the use in the bile duct and may prevent cholecystitis without disturbance of bile flow in a pig model. Human studies are needed to validate the current findings and to evaluate the long-term outcome in bile duct as well as pancreatic duct stricture.

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Disclosures Young Sik Woo, Kwang Hyuck Lee, Jong Kyun Lee, Dong Hyo Noh, Joo Kyung Park, Kyu Taek Lee and Kee-Taek Jang have no conflicts of interest or financial ties to disclose.

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