Journal of Industrial Microbiology (1995) 15, 152–155

Bacterial biofilm and clogging of biliary stents

JJY Sung

Department of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

Endoscopic biliary stenting has become a standard palliative treatment for obstructive jaundice due to malignancies of the pancreas and the hepatobiliary system. Despite the high initial success rate in achieving biliary drainage, durable endoscopic stenting has been limited by the clogging of biliary stents, usually after 4–5 months, due to formation of an adherent bacterial biofilm. Various methods have been investigated for the prevention of bacterial adhesion and prolongation of stent patency. These include: 1) prophylactic use of antimicrobial agents and bile salts; 2) testing of new stent material and new designs for these biliary stents; and 3) the recent introduction of self-expandable metal stents. Each method has its own merits as well as specific problems. This article reviews the pathogenesis of biofilm formation on the biliary stents and the latest status of research in avoiding the problem of stent occlusion.

Keywords: biliary stents; clogging; bacterial biofilm

Introduction

Malignant tumours causing biliary obstructions are associated with high morbidity and mortality. The annual incidence of pancreatic cancer in the United States is approximately 28 000 cases [42]. Malignancies of the gallbladder and the bile ducts are relatively few with an annual incidence of about 25% that of primary pancreatic cancer [28]. The rich lymphatic network and generous blood supply of the hepatobiliary tract assure early spread of these tumours. As a result, malignant tumours of the pancreas and the bile ducts are usually silent until a relatively late stage of the disease when curative surgery becomes impossible. Since the first endoscopic transpapillary insertion of biliary stents was performed in 1979 [30], this procedure has quickly become a standard method of palliation for pancreaticobiliary malignancies causing obstructive jaundice [4,19] (Figure 1). The success rate for endoscopic stenting exceeds 90% and initial procedure-related complications are rare [4,15,19]. When endoscopic stenting was compared with bypass surgery, the two modes of treatment were found to have comparable survival rate and quality of life [1]. Since it does not involve puncturing the liver, endoscopic stenting is also considered safer than percutaneous stenting [33]. Unfortunately, the plastic stents in current use have a strong tendency to clog, resulting in recurrence of jaundice, chills, fever and abnormalities of liver function. The median interval for a 10-French gauge (Fr) stent to remain patent is only 4–5 months [4,15,19]. Replacement with a new stent can resolve the problem, but only temporarily, until the lumen of the endoprosthesis is clogged again after a short duration. Clogging of a biliary stent thus poses a limitation to its use. Formation and growth of bacterial biofilm plays a central role in stent clogging [24,32]. In the last decade, various methods have been investigated to prevent, or at least to delay, the formation of adherent biofilm with the goal of prolonging the life of biliary stents. This article intends to review the present status of research in this area.

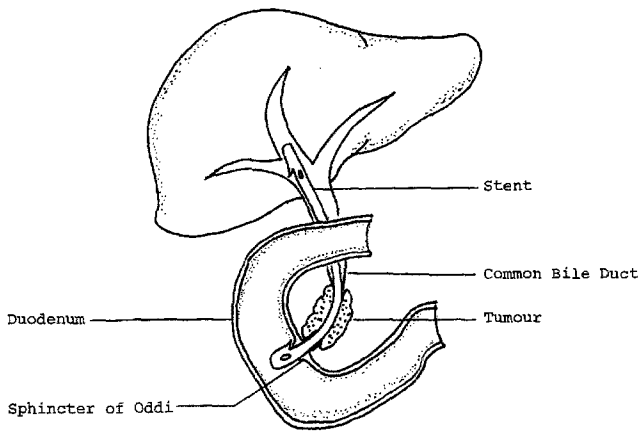

Figure 1 A line drawing of a biliary stent inserted for the palliation of tumour in the distal common bile duct causing obstruction of bile flow

Correspondence: JJY Sung, Department of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

Microbial ecology of the biliary tract

The biliary tract of a healthy individual does not harbour any microorganisms [6,10]. The sphincter of Oddi, which is located at the junction between the biliary tract and the duodenum (Figure 1), serves as a mechanical barrier to minimize bacterial invasion from duodenal reflux [37]. A daily bile flow of 800–1000 cc and the continuous secretion of mucus from the biliary epithelium ensure that microbes do not adhere to the biliary mucosa. Nevertheless, there are transient excursions of bacteria through the biliary system. When a foreign body is surgically implanted into the gallbladder, a bacterial biofilm forms on the foreign surface after 6–12 weeks [38]. The implant provides a favourable substratum for bacterial adhesion and proliferation, trapping bacteria passing through the biliary tract. Microorganisms invade the biliary tract via two major routes. First, bacteria from the lower gastrointestinal tract invade the biliary system via the portal circulation [41]. As the tight junctions between hepatocytes breaks down and Kupffer cell

functions are impaired with elevated intra-biliary pressure, the entry of bacteria via this hematogenous route is facilitated in obstructive jaundice. On the other hand, in patients who had undergone surgical sphincterotomy or endoscopic stenting, the function of the sphincter of Oddi as a mechanical barrier is disrupted and duodenal-biliary reflux is inevitable, leading to ascending infection of the biliary system [11,16,35].

Formation of bacterial biofilm results in stent clogging

A clogged biliary stent contains inspissated bile debris with a high protein content. Microcolonies of bacteria admixed with deconjugated bilirubin, protein and amorphous material are found under electron microscopy [24,32,43]. Based on the morphological similarities between the sludge material causing stent clogging and brown pigment biliary stones, we hypothesized that the two conditions have a similar pathogenic mechanism [36]. Bacterial β-glucuronidase and phospholipase of biliary pathogens deconjugate bilirubin-diglucuronide and lecithin in the bile, leading to the precipitation of calcium bilirubinate and calcium salts of fatty acids. With time, the biofilm grows as progressive agglomeration of bile sediment and biliary sludge form, resulting in occlusion of the stent lumen. The first step in the pathogenesis of stent occlusion is therefore adhesion of bacteria on the stent surface. A layer of protein was detected on the surface of the stent which was considered an important surface conditioning protein for bacterial adhesion [18]. However, the origin of this protein is unkown and its chemical nature remains to be elucidated. Defects introduced during the manufacture of biliary stents such as irregular inner surface and badly constructed side-holes could facilitate bacterial colonization [2,8]. New stent materials and different designs of the stents have thus been put forward (see below). The importance of β-glucuronidase production of biliary pathogens in the formation of biliary sludge has been debated. In an *in vitro* study, the amount of sludge formed by β-glucuronidase-producing bacteria was no more than that by non-enzyme producing bacteria [9].

Antibiotics and other antimicrobial agents

As clogging of biliary stents is a result of bacterial colonization and biofilm formation, it is logical to keep the biliary tract 'sterile' with antimicrobial agents. In the earliest report, aspirin (an anti-prostaglandin to reduce mucin secretion) and doxycycline (an antimicrobial to suppress bacterial growth) were given to a group of 60 patients who needed biliary stenting [29]. Unfortunately, doxycycline is not the right antibiotic to test since most biliary pathogens are gram-negative coliforms. A high dropout rate and short follow-up contributed to the failure of the study to confirm the benefit of antibiotic treatment. Recently, Libby *et al* added a sub-bactericidal dose of ciprofloxacin to *E. coli* infected bile and perfused polyethylene stents for 24 h in an *in vitro* system. Bacterial adherence to polyethylene stents was significantly reduced [25]. The decreased bacterial adherence was attributed to the change of bacterial surface characteristics, as shown by scanning electron microscopy. In an animal study, treatment with ciprofloxacin alone significantly prolonged the median stent patency from 17 to 62 days [26]. However, the benefits of prophylactive antibiotic treatment in this animal study have not been translated into clinical benefit. Ghosh *et al* used a cyclical antibiotics regimen (composed of ampicillin, metronidazole and ciprofloxacin) together with ursodeoxycholic acid (a choleretic agent), and found no improvement in the duration of stent patency [14]. In their study, however, antibiotics and ursodeoxycholate were commenced two weeks after endoscopic stenting. Once the first layer of bacterial biofilm has established itself on the stent surface, it would be more difficult to eradicate the biomass [3]. The use of long-term antibiotic therapy has always raised the concern of cultivating resistant bacterial strains as well as producing untoward effects by altering the bowel flora. Coating the plastic surface with a biocide could theoretically avoid these problems. Silver-coated polyurethane has been tested *in vitro* and showed a dose-related reduction in bacterial adherence by 10–100 fold [23].

New stent materials

Polyethylene, polyurethrane and Teflon™ are the most common materials for biliary stents. Less sludge formed on the surface of Teflon than on polyethylene and polyurethane stents perfused with infected bile [2,8]. Among these three materials, Teflon has the lowest coefficient of friction [17] implying that smoothness of surfaces directly affects adherence of bacteria. At least two new polymers have come into the market in recent years, both claiming to have an 'ultrasmooth' surface. Vivathane™, a cross-linked urea polymer with a critical surface tension approaching zero, is claimed to have no detectable surface irregularity at 2000 times magnification under scanning electron microscopy [27]. Hydromer™, a hydrophilic polymer (poly-*N*-vinylpyrrolidone or PVP), is coated on polyurethane which also produced a smooth texture to the stent surface [20]. Both products reduce bacterial adhesion in the infected bile perfusion experiments and thus prevent development of a bacterial biofilm. Although *in vitro* data on vivathane and hydromer seems promising, they have not been tested in clinical trials to confirm their superiority.

Bile salt

Bile salts have potent antibacterial activities which contribute to the sterility of the biliary system. When the antibacterial activity of bile salts with different hydrophobicities was tested, hydrophobic bile salts (eg deoxycholate) had more profound bactericidal effects on common biliary pathogens than the hydrophilic bile salts (eg taurocholate) [39]. Electrostatic interactions and surface hydrophobicity are crucial factors affecting bacterial adhesion [21]. Indeed, hydrophobic bile salts are potent inhibitors of adhesion of biliary pathogens on stent material [40]. Deoxycholate and taurodeoxycholate, even at sub-bactericidal levels, reduce bacterial adhesion on plastics by 100–1000 fold. It is an

attractive idea to develop an orally absorbable hydrophobic bile salt that recycles in the bile to prevent adhesion of bacteria on the biliary stent. A major problem with the use of hydrophobic bile salts is the cytopathic effects of these compounds which cause gastrointestinal upset. Bile salts such as ursodeoxycholate with greater hydrophilicity are better tolerated but have little effect on bacterial adherence [12]. The potential role of ursodeoxycholate, chenodeoxycholate or combination therapy in the prevention of stent blockage deserves further investigation.

New designs for biliary stents

One of the most important improvements in stent patency was made by using stents with a larger luminal diameter. Clinical study has shown, with convincing results, that a 10-Fr stent drains significantly better than the original 8-Fr stent [31]. However, the use of still larger stents is limited by the size of the endoscope channel. It is not feasible to place plastic stents with outer diameter greater than 12 Fr and the mean time-to-occlusion of these stents remains at 5–6 months [5]. Recently, self-expanding metal stents with a maximum diameter of 7–10 mm have significantly prolonged median stent patency [7,22]. The much bigger lumen of the fully deployed metal stent is undoubtedly useful in keeping the drainage of bile even with biofilm and sludge formation. Several investigators have pointed out that sludge formation in plastic stents accumulates mainly around the side holes [2,8]. The detrimental effect of the side-holes seems to outweigh the effect of stent material [2]. *In vitro* and *in vivo* studies have demonstrated that, by omitting side-holes in polyethylene stents, a reduction in the amount of sludge formed [2,8]. However, in our study of patients randomized to receive polyethylene stents with or without side-holes, the median time before total occlusion and the weight of encrustation formed did not show a significant difference between the two groups [34]. Furthermore, the partial thickness flaps in the stents without side-holes gave weaker anchorage support in the bile duct. Migration of stents into the duodenum after implantation has been reported, resulting in early drainage failure [34]. As ascending infection from duodenal-biliary reflux is an important route of acquiring infections, another modification of the design of biliary stents is placing the whole stent in the common bile duct above the sphincter of Oddi, preserving the function of the sphincter as a mechanical barrier. Animal studies have shown that stents placed above the sphincter have a longer duration of patency [13,35]. The clinical advantage of these non-protruding stents needs to be substantiated by clinical trials.

Conclusion

Biliary stents inserted for inoperable cancer of the hepatobiliary system are clogged by formation of bacterial biofilm 4–6 months after implantation. To prevent, or delay, the stent blockage, various methods have been tried. These include the use of antimicrobial agents and bile salt, the development of new stent materials and new designs for biliary stents. Most of these methods are still in experimental stages and none has proven clinical advantage in the prevention of biofilm formation. Until an effective method of preventing bacterial adhesion is achieved, the most promising result arises from using stents of a larger diameter, especially the self-expanding metal stents.

References

1 Anderson JR, SM Sorensen, A Kruse *et al.* 1989. Randomized trial of endoscopic endoprosthesis versus operative bypass in malignant obstructive jaundice. Gut 30: 1132–1135.
2 Coene PPLO, AK Greon, J Cheng *et al.* 1990. Clogging of biliary endoprosthesis: a new perspective. Gut 31: 913–917.
3 Costerton JW, Z Lewandowski, D DeBeer *et al.* 1994. Biofilms, the customized microniche. J Bacteriol 176: 2137–2142.
4 Cotton PB. 1990. Management of malignant bile duct obstruction. J Gastroenterol Hepatol 1: 63–77.
5 Cotton PB. 1990. Critical appraisal of therapeutic endoscopy in biliary tract disease. Ann Rev Med 41: 211–222.
6 Csendes A, M Fernandez and P Uribe. 1975. Bacteriology of the gallbladder bile in normal subjects. Am J Surg 129: 629–631.
7 Davids PHP, AK Groen, EAJ Rauws *et al.* 1992. Randomized trial of self-expanding metal stents versus polyethylene stents for distal malignant biliary obstruction. Lancet 340: 1488–1492.
8 Dowidar N, HJ Kolmos, H Lyon *et al.* 1991. Clogging of biliary endoprosthesis: a morphologic and bacteriologic study. Scand J Gastroenterol 26: 1137–1144.
9 Dowidar N, HJ Kolmos and P Matzen. 1991. Experimental clogging of biliary endoprosthesis: role of bacteria, endoprosthesis material and design. Scand J Gastroenterol 27: 77–80.
10 Dye M, A MacDonald and G Smith. 1979. The bacterial flora of the biliary tract and liver in man. Br J Surg 65: 285–287.
11 Feretis CB, CT Contou, AJ Manouras *et al.* 1984. Longterm consequences of bacterial colonization of the biliary tract after choledochotomy. Surg Gynecol Obstet 159: 363–366.
12 Galle P, L Theilmann, R Raedsch *et al.* 1990. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human hepatocytes. Hepatology 12: 486–491.
13 Geoghegan JG, MS Branch, JW Costerton *et al.* 1991. Biliary stent occlude earlier if the distal tip is in the duodenum in dogs. Gastroint Endosc 37: A257.
14 Ghosh S and KR Palmer. 1993. Prophylaxis of biliary stent blockage using cyclical antibiotics and ursodeoxycholic acid. Gastroenterology 104: A361.
15 Gilbert DA, AJ DiMarino Jr, DM Jensen *et al.* 1992. Status evaluation: biliary stents. American Society for Gastrointestinal Endoscopy. Technology Assessment Committee. Gastroint Endosc 38: 750–752.
16 Gregg JA, PD Girolami and DL Carr-Lock. 1985. Effects of sphincteroplasty and endoscopic sphincterotomy on the bacteriologic characteristics of the common bile duct. Am J Surg 149: 668–671.
17 Grimm H and N Soehendra. 1989. Endoscopic biliary drainage (Hamburg). In: Hepatobiliary and Pancreatic Malignancies: Diagnosis, Medical and Surgical Management (Lygidakis NJ, Tytgat GNJ, eds), pp 418–425, Thieme, New York.
18 Groen AK, T Out, K Huibregtse *et al.* 1987. Characterization of the content of occluded biliary endoprosthesis. Endoscopy 19: 57–59.
19 Huibregtse K, RM Katon, PP Coene *et al.* 1986. Endoscopic palliative treatment of pancreatic cancer. Gastroint Endosc 32: 334–338.
20 Jansen B, LP Goodman and D Ruiten. 1993. Bacterial adherence to hydrophilic polymer-coated polyurethane stents. Gastroint Endosc 39: 670–673.
21 Klotz SA. 1990. Role of hydrophobic interaction in microbial adhesion to plastics used in medical devices. In: Microbial Cell Surface Hydrophobicity (Doule RJ, Rosenbeg M, eds), pp 107–136, American Society for Microbiologists, Washington.
22 Knyrim K, HJ Wagner, J Pausch *et al.* 1993. A prospective, randomized, controlled trial of metal stents for malignant obstruction at the commmon bile duct. Endoscopy 25: 207–212.
23 Leung JWC, GTC Lau, JY Sung *et al.* 1992. Decreased bacterial adherence to silver-coated stent material: an *in vitro* study. Gastroint Endosc 38: 338–340.
24 Leung JWC, TKW Ling, KL Kung *et al.* 1988. The role of bacteria in blockage of biliary stent. Gastroint Endosc 33: 602–607.

25 Libby ED, A Coimbre and JWC Leung. 1994. Early treatment with antibiotics prevent adherence of biliary biofilm. Gastoint Endosc 40: A744.
26 Libby ED, D Morck, S McKay *et al.* 1994. Ciprofloxacin prevents stent blockage in an animal model. Gastroenterol 106: A346.
27 McAllister EW, LC Carey, PG Brady *et al.* 1993. The role of polymeric surface smoothness of biliary stents in bacterial adherence, biofilm deposition and stent occlusion. Gastroint Endosc 39: 422–425.
28 Roslyn JJ. 1992. Cancer of the gallbladder and bile ducts. In: Liver and Biliary Diseases (Kaplowitz N, ed), pp 658–672, Williams & Wilkins, Baltimore, Maryland.
29 Smit JM, MM Out, AK Groen *et al.* 1989. A placebo-controlled study on the efficacy of aspirin and doxycycline in preventing clogging of biliary endoprosthesis. Gastroint Endosc 35: 485–489.
30 Soehendra N and V Reynders-Frederix. 1980. Palliative bile duct drainage: a new endoscopic method of introducing a transpapillary drain. Endoscopy 12: 8–11.
31 Speer A, PB Cotton and K MacRea. 1988. Endoscopic management of malignant biliary obstruction: stents of 10 French gauge are preferable to stents of 8 French gauge. Gastroint Endosc 34: 412–417.
32 Speer A, PB Cotton, J Rode *et al.* 1988. Biliary stent blockage with bacterial biofilm: a light and electron microscopy. Ann Intern Med 34: 19–22.
33 Speer A, PB Cotton, RCG Russell *et al.* 1987. Randomized trial of endoscopic versus percutaneous stent insertion in malignant obstructive jaundice. Lancet ii: 57–62.
34 Sung JY, SCS Chung, CP Tsui *et al.* 1994. Omitting side-holes in biliary stents does not improve drainage of the obstructed biliary system: a prospective randomized trial. Gastroint Endosc 40: 321–325.
35 Sung JY, JWC Leung, EA Shaffer *et al.* 1992. Ascending of the biliary tract after surgical sphincterotomy and biliary stent. J Gastroenterol Hepatol 7: 240–245.
36 Sung JY, JWC Leung, EA Shaffer *et al.* 1993. Bacterial biofilm, brown pigment stone and blockage of biliary stents. J Gastroenterol Hepatol 8: 28–34.
37 Sung JY, ME Olson, JWC Leung *et al.* 1990. The sphincter of Oddi is th boundary of bacterial colonization of the gastrointestinal and the biliary system. Micro Ecol Health Dis 3: 199–207.
38 Sung JY, ME Olson, JWC Leung *et al.* 1991. The demonstration of transient bacterbilia by foreign body implantation. Dig Dis Sci 36: 943–948.
39 Sung JY, EA Shaffer and JW Costerton. 1993. Antibacterial activity of bile salts against common biliary pathogens: effects of hydrophobicity of the molecule and in the presence of phospholipids. Dig Dis Sci 38: 2104–2112.
40 Sung JY, EA Shaffer, K Lam *et al.* 1994. Hydrophobic bile salt inhibits bacterial adhesion on biliary stent material. Dig Dis Sci 39: 999–1006.
41 Sung JY, EA Shaffer, ME Olson *et al.* 1991. Bacterial invasion of the biliary system by way of the portal venous system. Hepatology 14: 313–317.
42 Warshaw A and C Fernandez-Del Castillo. 1992. Medical progress: pancreatic carcinoma. N Engl J Med 326: 455–465.
43 Wosiewitz U, B Schrameyer and L Safrany. 1985. Biliary sludge: its role during bile drainage with an endoprosthesis. Gastroenterology 88: 1076–1081.