Silver-nanoparticle-coated biliary stent inhibits bacterial adhesion in bacterial cholangitis in swine

Wei Wen, Li-Mei Ma, Wei He, Xiao-Wei Tang, Yin Zhang, Xiang Wang, Li Liu and Zhi-Ning Fan

Nanjing, China

BACKGROUND: One of the major limitations of biliary stents is the stent occlusion, which is closely related to the overgrowth of bacteria. This study aimed to evaluate the feasibility of a novel silver-nanoparticle-coated polyurethane (Ag/PU) stent in bacterial cholangitis model in swine.

METHODS: Ag/PU was designed by coating silver nanoparticles on polyurethane (PU) stent. Twenty-four healthy pigs with bacterial cholangitis using Ag/PU and PU stents were randomly divided into an Ag/PU stent group (n=12) and a PU stent group (n=12), respectively. The stents were inserted by standard endoscopic retrograde cholangiopancreatography. Laboratory assay was performed for white blood cell (WBC) count, alanine aminotransferase (ALT), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) at baseline time, 8 hours, 1, 2, 3, and 7 days after stent placements. The segment of bile duct containing the stent was examined histologically *ex vivo*. Implanted biliary stents were examined by a scan electron microscope. The amount of silver release was also measured *in vitro*.

RESULTS: The number of inflammatory cells and level of ALT, IL-1β and TNF-α were significantly lower in the Ag/PU stent group than in the PU stent group. Hyperplasia of the mucosa was more severe in the PU stent group than in the Ag/PU stent group. In contrast to the biofilm of bacteria on the PU stent, fewer bacteria adhered to the Ag/PU stent.

CONCLUSIONS: PU biliary stents modified with silver nanoparticles are able to alleviate the inflammation of pigs with bacterial cholangitis. Silver-nanoparticle-coated stents are resistant to bacterial adhesion.

(*Hepatobiliary Pancreat Dis Int 2016;15:87-92*)

KEY WORDS: biliary stent; silver nanoparticles; endoscopic retrograde cholangiopancreatography; bacterial cholangitis

Introduction

It has been more than 30 years since endoscopic biliary stenting was as a standard palliative treatment for obstructive jaundice due to malignant or benign obstruction of bile tracts.[1] Many studies[2-7] have shown that metal biliary stents may offer better palliation and longer patency than plastic stents in patients with malignant biliary obstruction. However, compared to plastic stent, the major disadvantages of metal stents are higher cost and difficult to reposit once deployed. Therefore, the metal stents may be not suitable for benign strictures.[8]

The occlusion of plastic stents remains a major problem in this treatment.[9] It is believed that microbial biofilms resulted from bacterial colonization and sludge deposition are closely associated with biliary stent clogging.[10-12] Antibiotic prophylaxis has been employed to reduce the risk of biliary bacterial colonization.[13] But the antibiotic treatment sometimes leads to allergic reactions (including anaphylaxis) and development of bacterial resistance, and increases costs of medical care. Hence, it is essential to design a new plastic stent to prevent bacterial colonization and biofilm formation.

Silver (Ag) nanoparticles have antimicrobial properties and have been seen as a candidate for antibacterial coating of stents.[14] Coatings using silver salts implanta-

Author Affiliations: Department of Digestive Endoscopy Center, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China (Wen W, Ma LM, Tang XW, Wang X, Liu L and Fan ZN); Nanjing Medical University, Nanjing 210029, China (Wen W); Department of Gastroenterology, The 81th Hospital of PLA, Nanjing 210002, China (Wen W); Department of Gastroenterology, Changzhou First People's Hospital, Third Affiliated Hospital of Suzhou University, Changzhou 213003, China (He W and Zhang Y)

Corresponding Author: Zhi-Ning Fan, MD, PhD, Department of Digestive Endoscopy Center, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China (Tel/Fax: +86-25-58509931; Email: fanzhining@njmu.edu.cn)

The abstract of this study was presented as a poster at the Asia Pacific Digestive Week (APDW 2013), which was held in Shanghai, China on September 21-24, 2013.

doi: 10.1016/S1499-3872(15)60410-6
Published online September 17, 2015.

tion of metallic silver have been devised.[15, 16] However, they have shown disappointing antibacterial ability because of obliteration or inactivation of the silver coating by blood plasma.

In an attempt to address these shortcomings while taking advantage of the broad antimicrobial spectrum of silver, we designed an Ag nanoparticle-impregnated polymer coating on plastic stents. The present study reports the antimicrobial activity of Ag nanoparticles in pig models and their release *in vitro*.

Methods

Creation of silver-nanoparticle-coated biliary stent

Biliary stents modified with Ag nanoparticles (Ag/PU stents) were prepared by coating Ag/PU composite suspension on polyurethane (PU) biliary stents. The Ag/PU composite suspensions were prepared by *in situ* reduction method. Firstly, 0.5 mL $AgNO_3$ absolute ethanol solution (1 mmol/L) was mixed with 50 mL tetrahydrofuran (THF) solution containing 5 g PU to obtain a PU/$AgNO_3$ solution in THF/ethanol. Then 0.5 mL freshly prepared $NaBH_4$ ethanol solution (0.02 mol/L) was added to the above PU/$AgNO_3$ solution with vigorous stirring to reduce Ag^+ to Ag nanoparticles. The bright-brown suspension was obtained after the reaction was completed. This *in situ* reduction method is able to obtain Ag/PU composite without aggregation of nanoparticles.

Then the Ag/PU composite suspension was cast on PU stent with outer diameter of about 8 mm, wall thickness of 0.3 mm, and length of 40 mm. The PU stent was immersed into the Ag/PU suspension for 10 seconds and slowly pulled out. After the fast evaporation of THF solvent, Ag/PU coating was formed on the PU stent. Because THF can partially dissolve the surface of the PU stent, the Ag/PU coating can be firmly attached to the surface of the PU stent. The control PU biliary stent and Ag/PU biliary stent are shown in Fig. 1. Brown color indicates the presence of Ag nanoparticles about 20 nm in diameter.

Animal preparations

Healthy pigs weighing from 19 to 24 kg (mean 22 kg) were used in the experiments. The experimental procedures were approved by the Animal Use and Care Committee of Nanjing Medical University, China. All the pigs were fasted for two days before the experiments. Anesthesia was induced with 3% pentobarbital by peritoneal injection, followed by an intramuscular injection of 250 mg of ketamine hydrochloride. Cardiac and respiratory parameters were recorded at baseline and 8 hours, 1, 2, 3, and 7 days after the placement of the stent.

After the pigs were anesthetized in the left lateral position, e-duodenoscopy was inserted through the esophagus, stomach into the duodenum. A small plastic tube was passed through the duodenoscope into the ampulla. Then 1.0 mL (15-18×10^8/mL) of *E. coli* (model ATCC 25922) solution was injected into the bile duct. The temporary obstruction of the nipple was solved with saline injection (Fig. 2).

Interventions

After the success of modeling (about 8 hours later), either PU stents or Ag/PU stents were intervened in 12 pigs with bacterial cholangitis respectively through an endoscope. The guide wire was sent to the duodenal papilla within the conveyor, then a stent was sent to the bile duct by the wire. Two pigs of each group were sacrificed at days 1 and 7, respectively, to observe bacterial adhesion on the surface of the stent. All the animals were killed by euthanasia.

Serological examination

Blood samples of the thoracic vein of each pig were taken for analysis of white blood cell (WBC) count at 8, 24, 48, 72 hours, and 7 days after modeling. The samples were partly centrifuged for analyzing aspartate aminotrans-

Fig. 1. Control PU biliary stent (upper) and Ag/PU biliary stent (lower).

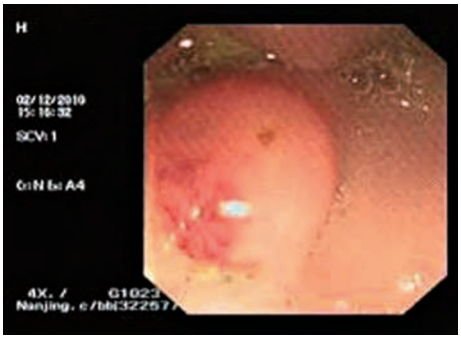

Fig. 2. The temporary obstruction of nipple with saline injection.