Repair and reconstruction of common bile duct by poly(lactide) stent

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Abstract
To investigate the effect of repair of bioabsorbable poly(lactide) (PLA) biliary stent in common bile duct (CBD) transection injury in canine prior to the clinical application. Circular tubing CBD stent was prepared by melt extraction technique using PLA. A transection incision was made on CBD of the normal canine, and then closed the incision with laser welding followed the implantation of PLA tubular stent into it. The stent was obtained to determine degradation of PLA in vivo at postoperative week 1, 4, and 12, respectively. The changes of outer diameter and burst pressure of CBD were investigated. Furthermore, serum liver enzyme values and CBD histopathological analysis were examined in the animals. The results noted that the polymer stent exhibited the same biomedical functions as T tubes and no significant tissue response. Therefore, biodegradable PLA stent matches the requirements in repair and reconstruction of CBD to support the duct, guide bile drainage and reduce T-tube-related complications.

Keywords: biliary stent; biodegradable polymer; poly(lactide); medical devices

INTRODUCTION
Bioabsorbable polymer, especially aliphatic polyesters, i.e., poly(lactic acid) (PLA), poly(glycolide) (PGA), poly(ε-caprolactone) (PCL) and their copolymer, poly(lactide-co-glycolide) (PLGA) and poly(lactide-co-caprolactone) (PLCL), have been used as medical devices approved by FDA because of their excellent mechanical and degradable properties, such as surgical sutures, drug carriers, tissue engineering scaffolds, and implants.1,2

Extrahepatic biliary duct injuries, including transaction, stricture or narrowing, and biliary leak, are well-known in upper abdominal surgeries including common bile duct exploration (CBDE), cholecystectomies and hepatic operations. Commonly, T-tube made of silicon rubber has been used in clinical practice to support common bile duct (CBD), ensure biliary decompression and create a track lined by granulation and fibrous tissue.3,4 However, several serious complications, e.g., biliary leakage, CBD stenoses, bile peritonitis, biliary fistula and electrolyte disurbances, due to T-tube implantation were caused to be as high as 15%.5,6 Various types of new stents were used to reduce such complications, such as, metal (Nitinol), plastic (polyethylene and polytetrafluoroethylene) or plastic-coated metal ones.7-13 However, these stents are usually non-bioabsorbable and then have to be withdrawn by a second procedure.

Biodegradable stent can offer advantages for the treatment of benign and malignant biliary diseases, including decreased biofilm formation and controlled anti-cancer drug delivery. In the past a few years, researchers studied CBD stent prepared by biodegradable materials. Freeman and Ginsberg described the safety and
usage of PLA CBD stent by endoscopy. In our previous research, degradation of PLGA CBD stent was determined in bile. The animal tests revealed that PLGA stent disappeared within 5 weeks in vivo. Tashiro et al. reported the feasibility of PLCL biliary tract device for porcine duct-to-duct biliary duct anastomosis. Furthermore, more experiments of biodegradable biliary tract stent need to do for clinic applications. In the present work, absorbable PLA tubular stent was prepared and placed into transaction injury of CBD in canine to explore the usefulness of stent in repair and reconstruction. The purpose of the study was to demonstrate the efficacy and tissue response of the PLA stent in mid- or long-term follow-up.

EXPERIMENTAL
Materials
Lactide (LA) was purchased from Purac, Holland and purified twice by recrystallization from ethyl acetate. Stannous octoate (Sn(Oct)2, 95%) was from Sigma-Aldrich. PLA was synthesized by bulk ring-opening copolymerization using Sn(Oct)2 as catalyst. Low molecular weight residuals were removed by a dissolution precipitation method in which chloroform and methanol were used as the solvent and precipitant, respectively. Then PLA was dried to remove solvent under reduce pressure for one week at room temperature.

Fabrications of CBD stent
PLA was extruded into circular tubing with a Model XSS-300 extruder (screw diameter 20 mm and length-to-diameter ratio 25) at 155 °C. The tubing was cut into CBD stent, which had an outer diameter of 10.0 mm, an inner diameter of 6.0 mm, a length of 50-60.0 mm.

Surgical procedure
The stent sterilized with 60Co radial and used for CBD implantation in canine. Nine mongrel dogs (weighting 15 to 20 kg) were housed in Jilin University Research Resources Center. They had free access to rodent diet and tap water. The University Animal Care and Use Committee approved all animal procedures. After anesthetization and standard laparotomy, the CBD was liberated, and a transection incision about one third of perimeter was made. A PLA stent was inserted into the lumen of CBD through the incision. Its position was properly adjusted to locate the incision in the middle of the stent. Then the stent was fixed on CBD by a suture and the incision was closed by a CO2 laser welding (Fig. 1).

Measurements
Three dogs were killed and stents were obtained from the CBD in week 1, 4, and 12 after surgical procedure, respectively. Degradation of the specimens was monitored by determining gross or microscopic inspection and molecular weight changes. Morphological changes of surface and cross-section were observed using scanning electron microscope (SEM, SS-550, Shimadzu) after gold-coating. The molecular weight was evaluated by size exclusion chromatography (SEC). A Waters 410 high pressure liquid chromatograph (HPLC) pump equipped with the columns of HT5, HT4
and HT3 in series was used for this purpose. The measurement was carried out at 25 °C using chloroform as mobile phase at a flow rate of 1.0 ml·min⁻¹ and polystyrene as a standard.

Function of CBD evaluation

The changes of CBD outer diameter in canine before and after surgical procedures were measured when PLA stent was inserted into billey tract and harvested at postoperative week 1, 4, and 12, respectively. To investigate the strength of dog’s bile duct, CBD burst pressure values were also recorded at the scheduled time with barometer.

Liver serum enzyme assay

Functions of biliary system and liver were monitored by examination of serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and γ-Glutamyltransferase (GGT) using biochemical analysis with those of before and after surgical procedures.

Histopathology analyses

Mid-portion of CBD of the dog was harvested at 1, 4 and 12 weeks after implantation, respectively. Tissue specimen was fixed in 2.5% glutaraldehyde for 30 min and dehydrated using graded alcohol series and dried. The specimen was embedded in paraffin and stained with hematoxylin and eosin (H&E), then observed by optical microscopy.

RESULTS AND DISCUSSION

Degradation of PLA stent in dogs

Biodegradable polymeric materials were developed rapidly in the late 20th century. The polymeric materials, including PLA, PGA and PCL are widely used in medical practices, like suture thread, in inner-fixation of bone fractures, tissue engineering scaffolds, and drug controlled release carriers. PLA has been known to degrade by simple hydrolysis of ester bonds and cleavage of chains into lactic acid, and finally are removed from the body by normal metabolic pathways. Prototype biodegradable stents were available for vascular or urologic usages, currently being exploded for biliary duct and gastrointestinal applications. According the literatures, PLA remained integrity in 6 months in CBD of animal studies and lost mass between 18 to 36 months.

In the present study, degradation of PLA was tested in bile (pH=7.2-7.6) in vivo. Gross appearances of the stents were illustrated in Fig. 2. The size and integrity of the device implanted in canine CBD were examined as a function of postoperative time. It was observed that there is no remarkable change of PLA stent in the first week. Then, color of the stent changed from initially translucent to white and opaque due to water absorption in week 4. But its strength and toughness was maintained. At the beginning of the 12 week, they began to be expanded and slightly deformed, but still allowed free drainage of bile.

The micrographs of PLA biliary duct stent in canine were shown in Fig. 3. The stent surfaces and cross-sections initially were smooth and nonporous (Fig. 3a and 3b). However, extensive micropores were observed during degraded process (Fig. 3b, c, d and 3e). Comparison of surface and cross-section of the samples, inner layer exhibited micropores of a broad size
than that of external. Pores in cross-section were larger than that of surface, due to self-catalyzed effect (Fig. 3c and e).

The weight average molecular weight (Mw) of PLA was about $2.6 \times 10^6$ g·mol$^{-1}$ while polymer was synthesized. Degradation occurred in the process of the stents and Mw decreased to $2.2 \times 10^6$ g·mol$^{-1}$. Reduction of molecular weight in CBD of canine was faster at the early stage of during the bile degradation (from $2.2 \times 10^6$ to $4.8 \times 10^5$ g·mol$^{-1}$ in week 4). From then on, the molecular weight reduction rate decreased slowly, especially during later stage (Fig. 4). Decrease of molecular weight meant polymer chain randomly broken, and macromolecules changed to low or small molecules.

Function of biliary duct
Bile leaks or fistulae of anastomosis are serious complications of hepatobiliary surgery. They may occur after surgical bile duct injury and penetrating or blunt trauma. It is critical to reduce the incidence of iatrogenic biliary leakage in operations. Among nine dogs, there is no biliary leakage was found during the experimental period. The results indicated PLA circle tubular stent can protect transection incision and prevent from bile outgoing. Furthermore, laser welding technique is a safe and effective method to suture incision.

The change of CBD outer diameter was demonstrated the CBD function. As seen as in Fig. 5. A slight increase of outer diameter in week 12 was observed comparing with that of original. No jaundice was seen in the experiments. The results suggested there is no remarkable biliary duct obstruction
occurred after insertion PLA device into CBD. PLA stent played an important effect to support biliary tract repair and bile drain. If the structure of the instrument is maintained for 3 to 5 months, biliary stricture may be prevented for a long time.\textsuperscript{17}

**Fig. 4.** Change of molecular weight of PLA stent in bile in canine.

**Fig. 5.** Change of outer diameter of common bile duct of canine before and after insertion.

**Fig. 6.** Burst pressure of CBD in canine at different periods postoperation in canine.

**Fig. 6** showed CBD burst pressure in different postoperative periods. From the data, the levels of CBD burst pressure were significantly increased from 30 KPa at the first week to 140 KPa at the 12th week postoperation, respectively. The results noted that the strength of biliary tract improved, thus, the CBD function was repaired and reconstructed.

Liver function measurements

The measurements of enzyme values, such as ALP, ALT and GGT, are sensitive to biliary tract stenosis, obstruction and cholestasis.\textsuperscript{8} Examination of liver serum enzyme suggested that there was initial significant impact of implant on liver function. As shown in **Figure 7**, the levels of serum enzyme in animals had increased after surgery procedure and reached peak value at week 2 postoperatively. The levels of ALP, ALT and GGT returned gradually to normal value by week 6 after transplantation. The similar results were reported by Zogrofokis et al.\textsuperscript{8} The temporary elevation of liver enzyme system after surgery demonstrated that it is useful to place and support the repair of CBD using stent.

Histopathology

Microscopic examinations were done on the specimens as described in Section 2.7. Histological results (**Fig. 8**) revealed that there was not significant integration or epithelial hyperplasia in the first week post-deployment stent. At the fourth week necropsy, most parts of inner membrane of incision were closed up. Chronic inflammation and cicatrisation in the incision were minor.
After 12 weeks, epithelium of the anastomosis in bile duct were repaired and arranged. Slight fibrosis were observed.

CONCLUSIONS

T-tubes are widely used in clinical practice to relieve CBD obstructions. However, T-tube-related complications, for examples, biliary leakage or fistula, water-electrolyte disturbance, and strictures are reported to be as high as 15%.\textsuperscript{,5,6,21} Many studies to decrease the incidence of complications were reported.\textsuperscript{22-27} As simple suturing often induces hemorrhage and may cause complications e.g. bile duct narrowing,\textsuperscript{28-30} it is necessary to insert a stent into CBD.

In this study, circular tubing PLA stents were prepared for repair of transaction injury of CBD in dogs. Biodegradation behaviors of PLA device, i.e., molecular weight and morphologies, were investigated during 3 months in vivo. The structures and functions of CBD and liver, such as the changes of outer diameters, burst pressures, liver serum enzyme level, and tissue response pre- and post-operation were also determined. The results revealed the safety and efficacy of reconstruction of CBD using PLA stent. The research also provided evidences for development biodegradable self-expanding digestive stent for gastroenterologic applications.

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