Original Article

Effects of Paclitaxel-conjugated N-Succinyl-Hydroxyethyl Chitosan Film for Proliferative Cholangitis in Rabbit Biliary Stricture Model

Tao Wang[1,2], Hao Zou[1], Yun-Xia Liu[3], Xiao-Wen Zhang[1,2]

[1]Department of Hepatopancreatobiliary Surgery, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, China
[2]Graduate Division, Kunming Medical University, Kunming, Yunnan 650500, China
[3]Basic Medical Division, Experiment Teaching Center, Kunming Medical University, Kunming, Yunnan 650500, China

Abstract

Background: Paclitaxel (PTX) could inhibit the growth of fibroblasts, which occurs in proliferative cholangitis and leads to biliary stricture. However, its use has been limited due to poor bioavailability and local administration for short time. This study designed and synthesized a new PTX-conjugated chitosan film (N-succinyl-hydroxyethyl chitosan containing PTX [PTX-SHEC]) and evaluated its safety and efficiency using *in vivo* and *in vitro* experiments.

Methods: The SHEC conjugated with PTX was confirmed by nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FT-IR) measurements. Drug releases *in vitro* and *in vivo* were determined using high-performance liquid chromatography. Cell viability *in vitro* was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Rabbit biliary stricture model was constructed. All rabbits randomly divided into five groups ($n = 8$ in each group): the sham-operated rabbits were used as control (Group A), Groups B received laparotomies and suture, Group C received laparotomies and covered SHEC suture without the PTX coating, Group D received laparotomies and covered PTX-SHEC suture, and Group E received laparotomies and 1000 μmol/L PTX administration. Liver function tests and residual dosage of PTX from each group were measured by enzyme-linked immunosorbent assay. Histological data and α-smooth muscle actin (SMA) immunohistochemical staining of common bile duct were examined.

Results: NMR and FT-IR indicated that PTX was successfully introduced, based on the appearance of signals at 7.41–7.99 ppm, 1.50 ppm, and 1.03 ppm, due to the presence of aromatic protons, methylene protons, and methyl protons of PTX, respectively. No bile leak was observed. The PTX-conjugated film could slowly release PTX for 4 weeks (8.89 ± 0.03 μg at day 30). The *in vitro* cell viability test revealed significantly different levels of toxicity between films with and without PTX (111.7 ± 4.0% vs. 68.1 ± 6.0%, $P < 0.001$), whereas no statistically significant difference was observed among the three sets of PTX-contained films (67.7 ± 5.4%, 67.2 ± 3.4%, and 59.1 ± 6.0%, $P > 0.05$). Histological examinations revealed that after 28 days of implantment, Groups D and E (but not Group C) had less granulation tissue and glandular hyperplasia in the site of biliary duct injury than Group B. The pattern was more obvious in Group D than Group E. Less α-SMA-positive cells were found in tissue from Groups D and E. Comparing with Group E, the liver function was improved significantly in Group D, including total bilirubin (2.69 ± 1.03 μmol/L vs. 0.81 ± 0.54 μmol/L, $P = 0.014$), alanine aminotransferase (87.13 ± 17.51 U/L vs. 42.12 ± 15.76 U/L, $P = 0.012$), and alkaline phosphatase (60.61 ± 12.31 U/L vs. 40.59 ± 8.78 U/L, $P < 0.001$).

Conclusions: PTX-SHEC film effectively inhibites the myofibroblast proliferation and extracellular matrix over-deposition during the healing process of biliary reconstruction. This original film might offer a new way for reducing the occurrence of the benign biliary stricture.

Key words: Biliary Stricture; Chitosan; Paclitaxel; Proliferative Cholangitis; Slow-Releasing

Introduction

Proliferative cholangitis (PC) could be triggered by damage to the bile ducts during surgery, trauma to the abdomen, or other diseases,[1] which occurs very frequently, and is the

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Address for correspondence: Dr. Xiao-Wen Zhang, Department of Hepatopancreatobiliary Surgery, The Second Affiliated Hospital of Kunming Medical University, 374# Dianmian Avenue, Kunming, Yunnan 650101, China
E-Mail: zhangxiaowenlu@hotmail.com

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main reason of biliary stricture. Biliary stricture is associated with the development of fibroblasts proliferation, collagen deposition, fibrosis, and narrowing of the bile duct lumen, representing an important cause of high morbidity and mortality of patients with biliary tract diseases.[2] Several treatment options are currently available for bile duct strictures including endoscopic, percutaneous balloon dilatation, and insertion of an endoprosthesis as well as surgery, of which surgery is crucial because it is necessary for patients failing to endoscopic therapy.[3] Nevertheless, restenosis could occur due to various subsequent biliary-enteric anastomosis after surgery.[4] An estimated 30% of patients have developed benign biliary strictures after their first surgery and required a second surgical treatment, and a small portion of patients developed biliary cirrhosis which required liver transplantation.[5] Reducing the occurrence rate of PC and subsequent fibrosis after surgery is a challenging problem.

Paclitaxel (PTX), an anti-fibrosis medication, has been introduced to reduce fibrosis on anastomotic wound healing after biliary reconstruction.[6] In previous studies, stricture was dilated using a PTX-eluting balloon or stent which received sustained clinical success.[7-9] However, this approach is imperfect and still needs to be improved because the PTX has limited access to the biliary stricture site across the blood-biliary barrier when administered systemically. Second, the efficiency could be reduced because of elution of PTX by bile. In addition, high concentration of PTX has a site effect, which means that it should be locally administrated. Finding a successful local delivery of PTX to the scar and slow sustained release is crucial.

Recently, several drug-loaded chitosan (CTS) films have emerged toward the development of a novel drug delivery system with high mechanical strength and low biodegradation rate.[10] CTS is one of these new types of biological material, characterized by certain advantages including nontoxic, low immunogenicity, antibacterial, and biodegradability and high biocompatibility.[11] It can also be easily secured by suturing at the site for sustained local delivery of coating agents without any displacement problems. Unfortunately, the drawback of CTS is poor solubility in water or organic solvents, which has limited its utilization.[12]

In this study, we developed a chemically modified CTS film-based local delivery system for sustained release of PTX to biliary stricture after implantation. We evaluated the safety and efficacy of the system *in vitro* and *in vivo*.

Methods

Ethical approval

All experimental procedures were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Ethics Committee of Animal Experiments of Kunming Medical University (Permit Number: 2014008). All efforts were made to minimize suffering.

Preparation and characterization of N-succinyl-hydroxyethyl chitosan containing paclitaxel films

A modified film, namely, N-succinyl-hydroxyethyl chitosan containing PTX (PTX-SHEC), was developed. The synthesized protocol is given in the Supplementary Methods. The hydroxyethyl chitosan (HEC) and SHEC in the absence of PTX were used as the controls. The effects of HEC, SHEC, and PTX-SHEC films were tested using nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FT-IR) measurements.

In vitro release of N-succinyl hydroxyethyl chitosan containing paclitaxel-conjugated membranes

PTX released from the polymer membrane was diluted in phosphate-buffered saline (PBS; pH 7.4) with 0.9% sodium chloride solution and 0.1% Tween 80 (w/v). The polymer membrane sample (1 cm^2) was placed in a centrifuge tube containing 3 ml of release medium. At predetermined time points (1, 2, 3, 5, 7, 14, and 30 days), release medium was extracted, and the same fresh PBS mentioned above was added. The previous steps were repeated for the collection of samples. The concentration of PTX released from polymer membrane was examined using high-performance liquid chromatography (HPLC). Two milliliters of dichloromethane was added and the mixtures were stirred for 5 min. Then, the supernatant was discarded. After dichloromethane was evaporated, the residue was dissolved in 1 ml of mobile phase. The analysis was performed using an Agilent 1100-HPLC system (Agilent Technologies, USA). The analytical column was C18 (4.6 mm × 150.0 mm). The system was equipped with autosampler and column oven set at room temperature. The mobile phase was a mixture of acetonitrile (58%), methanol (5%), and water (37%) used after filtration through 0.45 μm membrane filter. Injection volume was 10 μl. The flow rate was 1.0 ml/min. PTX was detected by detection of 230 nm absorption at a retention time of approximately 5 min. Under these conditions, the linear calibration curve of PTX was obtained in the concentration range 0.2–30.0 μg/ml ($r^2 > 0.99$).

In vitro toxicity study

A human cholangiocarcinoma cell line 1 was incubated in a 96-well culture plate (Nunc, Rochester, New York, USA) with 1×10^4 cells per well for 24 h at 37°C with 5% CO_2. The films of the same size were manufactured with different amounts of SHEC and PTX, as previously described. They were subsequently dissolved in dimethyl sulfoxide and were inserted into the wells of the plate. After 24 h of incubation, the numbers of viable cells were calculated using a Cell Proliferation Kit (MTT; Sigma-Aldrich, St. Louis, MO, USA) and measured at a wavelength of 570 nm.

Benign biliary stricture model in rabbit

Forty 3-month-old Japanese big-eared rabbits of both genders (Institute of Laboratory Animal Science, Kunming Medical University, Kunming, China) were fed *ad libitum* under the conditions of a temperature of 22°C and a relative humidity of 55%, with a 12-h diurnal rhythm. They

were conditioned for 1 week before the experiment. All rabbits with an average weight of 3.1 kg were cared for in compliance with the institutional guidelines and randomly divided into five groups as follows (n = 8 in each group): the sham-operated rabbits were used as controls (Group A), Groups B received laparotomies and suture, Group C received laparotomies and covered SHEC suture without the PTX coating, Group D received laparotomies and covered PTX-SHEC suture, and Group E received laparotomies and 1000 μmol/L PTX administration.

Rabbits were anesthetized with an intravenous injection of 3% pentobarbital sodium (2 ml/kg). An incision across rectus of the right upper abdomen was made, and common bile duct (CBD) was separated at the point of 1 cm away from the superior margin of the duodenum (the range of separation was within 1.5 cm). A suture of the lateral wall of CBD was made with the length of about a half of its circumference, using a 6-0 Polypropylene suture passing through all layers of the duct wall. Then, the PTX-SHEC-conjugated membrane (the size of membranes was approximately 1 cm^2) was wrapped outside of the local CBD and confirmed no bile leak at the CBD. Finally, the abdomen was closed. Postoperative anti-infection treatment was administered for 3 days.

Blood samples were collected from the marginal ear vein at 2 and 4 weeks after film implantment. Serum was separated immediately and preserved at −70°C. After a 4-week observation period, the experimental animals were sacrificed. The bile ducts were isolated and resected, and preserved in 100% formalin before pathologic examination.

In vivo drug release (rabbit serum analysis)

The amount of PTX released into the rabbit serum was measured by HPLC on days 0, 1, 3, 7, 14, and 28 after film implantment.

Follow-up parameters

Clinical observations

The incidence of bile leakage and animal mortality was investigated.

Histological parameters

The rabbits were sacrificed 4 weeks after the operation, and the segment of the bile duct was examined histologically. Hematoxylin and eosin (H&E) staining and Masson's staining were performed as previously described by Shi *et al*.[6] The sections were examined under a light microscope and graded in a blinded manner using a modified histological grading scale by Shi *et al*.[6] Collagen deposition and α-smooth muscle actin (SMA)-positive cell ingrowth were taken as the evaluating parameters. The positive intensity of α-SMA in vascular smooth muscle cells was used as the reference staining value.

Biological parameters

Liver function tests, including alanine aminotransferase (AST), total bilirubin (TBIL), and alkaline phosphatase (ALT), were performed before each surgery and at week 2 and 4 after bile duct injury.

Statistical analysis

All the data were presented as mean ± standard error (SE). The two-sample Student's t-test was conducted for comparison of each pair of samples, and one-way analysis of variance was used for comparing group means of the three groups. Analyses were conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Significant difference was considered at $P < 0.05$.

Results

Synthesis and characterization of N-succinyl-hydroxyethyl chitosan containing paclitaxel membrane

The synthetic procedures of PTX-SHEC are summarized in Figure 1a. The successful formation of PTX-SHEC was verified by ^{1}H NMR analysis [Figure 1b], based on the appearance of signals at 7.41–7.99, 1.50, and 1.03 ppm, due to the presence of aromatic protons, methylene protons, and methyl protons of PTX, respectively. The FT-IR spectra of SHEC and PTX-SHEC are shown in Figure 1c. Compared with the FT-IR spectrum of SHEC, the absorbance at 1728/cm and 1570/cm of PTX-SHEC indicated the presence of the ester and phenyl groups, respectively. The absorbance of C-O group at 1112/cm and 1043/cm increased, which confirmed the incorporation of PTX.

Drug release and cell toxicity *in vitro* and *in vivo*

The releases of PTX from 1 cm^2 of PTX-SHEC membrane were examined at 1, 2, 3, 5, 7, 14, and 30 days after incubation. The results of HPLC analyses showed that PTX has been continuously released from the membrane at least 30 days (8.89 ± 0.03 μg at day 30), and no burst release was observed during the first day [Figure 2a].

The *in vitro* cell viability test revealed significantly different levels of toxicity between films with and without PTX (111.7 ± 4.0% vs. 68.1 ± 6.0%, t = 10.550, $P < 0.001$), whereas no statistically significant difference was observed between the three sets of PTX-contained films (67.7 ± 5.4%, 67.2 ± 3.4%, and 59.1 ± 6.0%, F = 51.660, $P > 0.05$; Figure 2b), indicating that the SHEC film was safe *in vitro*.

We further analyzed the amount of PTX released into the rabbit serum over a duration of 4 weeks using HPLC. In all samples, the concentration of released PTX increased in the first 7 days and started to decrease at sometime between days 7 and 14. Released PTX was detectable in sample associated with high cross-linking degree PTX-SHEC (1:40 and 1:80) until day 28, but not in samples associated with low cross-linking degree PTX-SHEC (0 and 1:20; Figure 2c).

Postoperative recovery and complications following films insertion

No animal died after bile duct ligation. After surgery, no bile leak occurred. One rabbit in Group B died of wound dehiscence. The other rabbits had good postoperative courses after peritoneal drainage for 3–5 days.

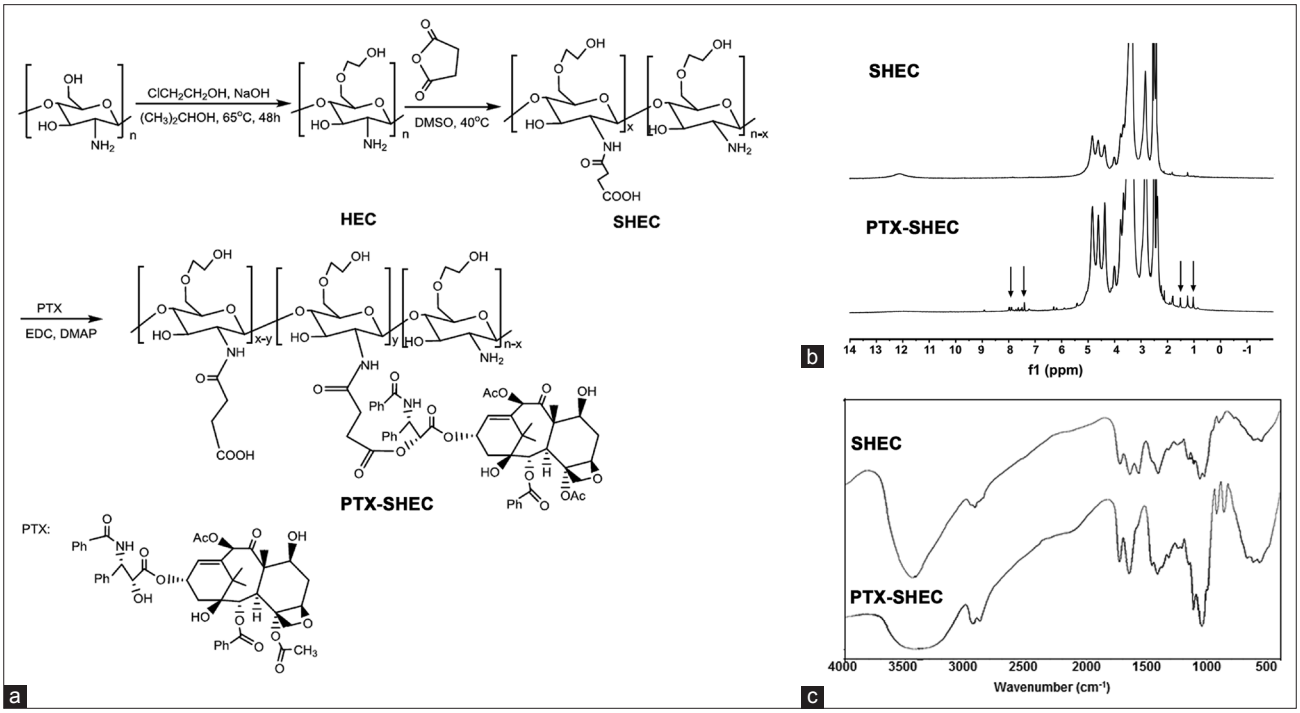

Figure 1: Synthesis and characterization of PTX-SHEC film. (a) Procedure for preparation of the PTX-SHEC-conjugated membranes. (b) ^{1}H NMR spectra of SHEC and PTX-SHEC. (c) FT-IR spectra of SHEC and PTX-SHEC. HEC: Hydroxyethyl chitosan; SHEC: N-succinyl-hydroxyethyl chitosan; PTX: Paclitaxel; EDC: 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride; DMAP: 4-dimethylaminopyridine; ^{1}H NMR: ^{1}H nuclear magnetic resonance; FT-IR: Fourier-transform infrared spectroscopy.

Histological changes

The effect of PTX-SHEC film on biliary stricture *in vivo* is shown in Figure 3. The obstruction and scars of the CBD were pointed with arrows [Figure 3a–3e], with gross dilatation proximal to the level of the previously placed suture. Up to 4 weeks after film placement in Group A, the bile duct at the separation site showed no stricture [Figure 3a]. However, in Groups B and C, pathologic specimen showed the formation of the scar at suture site away from the superior margin of duodenum and dilation of CBD above scar level [Figure 3b and 3c]. Compared with Group E [Figure 3e], CBD in Group D did not thicken and its wall had no abnormal change. The bile duct above the suture site revealed no dilatation [Figure 3d].

Microscopically, no proliferation was observed in Group A [Figure 3f]. H&E staining revealed deep epithelial-glandular proliferation, exuberant granulation tissue, inflammatory cell infiltration, and ductal fibrosis, with a loss of normal structure in Group B [Figure 3g] and Group C [Figure 3h]. In Groups D and E, we also observed lower degrees of ductal fibrosis [Figure 3i and 3j], especially in Group D.

The results of Masson's staining showed that when compared with Group A, submucosa in Group B was markedly thickened through fibrosis while the submucosal mucous glands were not visible [Figure 3k]. In addition, the superficial muscle layer was affected by fibrosis [Figure 3l]. A similar pattern could be found in Group C as well [Figure 3m]. In Group E, moderate submucosal fibrosis and abundant submucosal mucous glands were observed [Figure 3o], but only mild submucosal fibrosis in Group D [Figure 3n]. Despite the fact that the mild muscular fibrosis was developed, a proper muscle layer remained [Figure 3o].

We also examined the appearance of a myofibroblast marker, α-SMA. In the normal wall of bile duct in Groups A and D, the expression levels of α-SMA were low [Figure 3p and 3s]. The immunohistological staining of α-SMA showed that when compared with Group A, the number of myofibroblasts increased in Group B [Figure 3q] and Group C [Figure 3r]. Fewer α-SMA-positive cells were observed in Group E [Figure 3t], and even fewer in Group D [Figure 3s]. Consistent with the results from Masson's staining, PTX-SHEC film reduced the numbers of myofibroblasts. These results indicated that administration of PTX-SHEC film was more effective than pure PTX on benign biliary stricture.

Liver function changes

Changes of liver function often occurred in benign biliary stricture, with the elevated level of TBIL, liver transaminases including AST and ALT.[13] Thus, the liver function parameters between groups were assessed [Figure 4]. Compared with Group A, the levels of serum TBIL, AST, and ALT in Group B were significantly higher (or nearly so) (0.62 ± 0.17 μmol/L vs. 3.85 ± 2.32 μmol/L, $t = 3.927$, $P = 0.002$; 40.21 ± 10.62 U/L vs. 123.66 ± 23.95 U/L, $t = 9.009$, $P = 0.005$; and 38.71 ± 7.13 U/L vs. 84.67 ± 21.73 U/L, $t = 5.684$, $P < 0.001$, respectively) at 4 weeks. The administration of PTX-SHEC film in Group D significantly reduced the

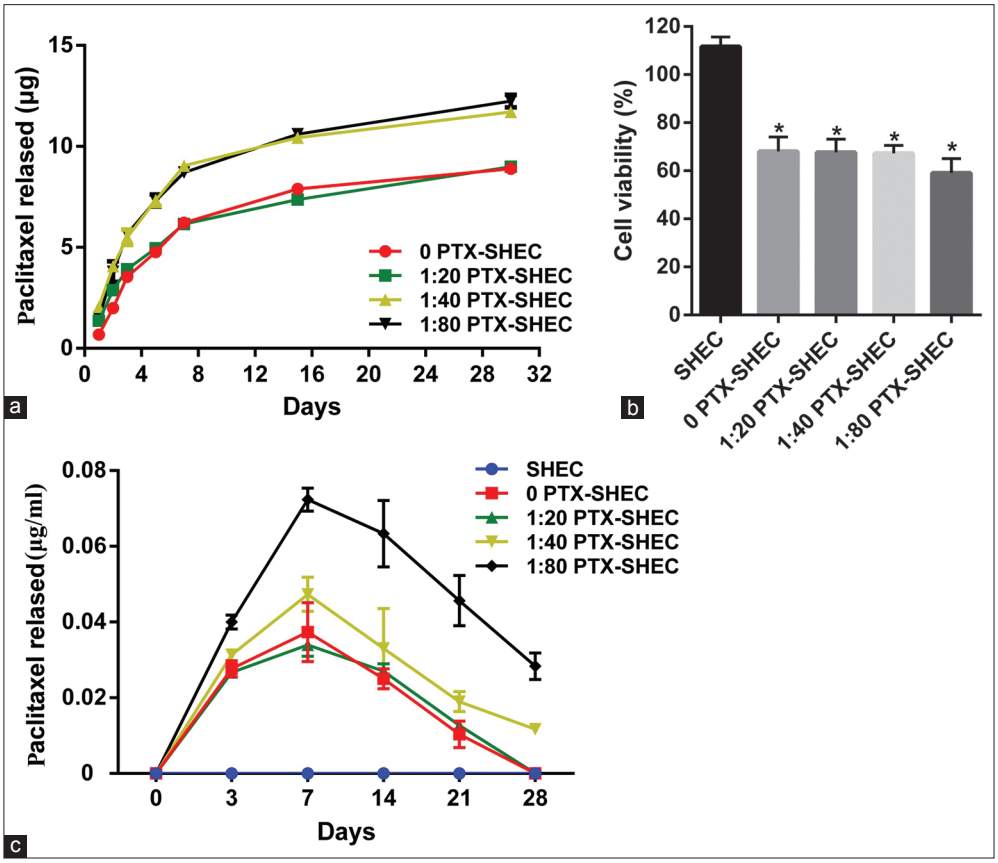

Figure 2: Drug release kinetics and toxicity of PTX-SHEC film. (a) *In vitro* release of PTX from PTX-SHEC membrane. (b) Cell viability in the human cholangiocarcinoma cell line-1 assessed by tetrazolium-based colorimetric MTT assay with varying concentrations of SHEC, showing a tendency to increased toxicity in the cancer cells with increasing concentrations of SHEC. (c) Mean serum concentrations of paclitaxel measured by high-performance liquid chromatography over a 28-day period in the four cross-linking degree groups (0 PTX-SHEC, 1:20 cross-linking degree PTX-SHEC; 1:40 cross-linking degree PTX-SHEC; and 1:80 cross-linking degree PTX-SHEC). *$P < 0.05$, compared with SHEC group. SHEC: N-succinyl-hydroxyethyl chitosan; PTX: Paclitaxel; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

levels of TBIL (0.81 ± 0.54 μmol/L, $t = 3.610$, $P < 0.001$), AST (42.12 ± 15.76 U/L, $t = 8.044$, $P < 0.001$), and ALT (40.59 ± 8.78 U/L, $t = 5.320$, $P < 0.001$) at 4 weeks, comparing with Group B. Significant reduction in levels of serum TBIL (2.69 ± 1.03 μmol/L, $t = 1.293$, $P = 0.217$), AST (87.13 ± 17.51 U/L, $t = 3.483$, $P = 0.004$), and ALT (60.61 ± 12.31 U/L, $t = 2.725$, $P = 0.016$) was also observed in Group E, compared with Group B. There was significant reduction of TBIL ($P = 0.014$), AST ($P = 0.012$), and ALT levels ($P < 0.001$) in Group D than those in Group E. These results indicated that PTX-SHEC film efficiently improved the liver function during PC.

Discussion

The management of benign bile duct stricture is always a challenge to the clinicians. Previous studies showed that PTX, an antineoplastic agent, could reduce the development of PC and subsequent bile duct stricture.[14,15] Unfortunately, poor solubility has limited its clinical usage.[16] In the last few years, great efforts have been made for development of new delivery systems for PTX, such as polymer conjugates, liposomes, polymeric micelles, parenteral emulsions, polymeric micro/nanoparticles, and water-soluble prodrugs.[17] For bile duct stricture, PTX-coated stents have been supported as effective delivery systems of relieving the obstruction. However, it is difficult to use stents participating in first intervention after liver transplantation or biliary injury. The stent implantation, being as a foreign material *in vivo*, could also result in adverse reactions including severe inflammatory response,[18] widespread epithelial necrosis,[19] and epithelial proliferation.[20] In this study, we developed a PTX-SHEC film for a better efficiency of preventing development of bile duct stricture.

CTS is a new type of biological material characterized by nontoxic, low immunogenicity, antibacterial, biodegradability, and biocompatibility. CTS also has good film reforming properties and cationic characteristic.[21] Nevertheless, CTS suffers a low solubility in water or organic solvents, which is a major drawback and limits its utilization. To enhance the hydrophilicity and biocompatibility of CTS, we developed a water-soluble derivative of CTS, i.e., HEC by a chemical method. HEC is characterized by high solubility in water, eminent mechanical performances, good flexibility, and no cytotoxicity.[22,23] A membrane of HEC is a good implant agent and can be easily applied to biliary

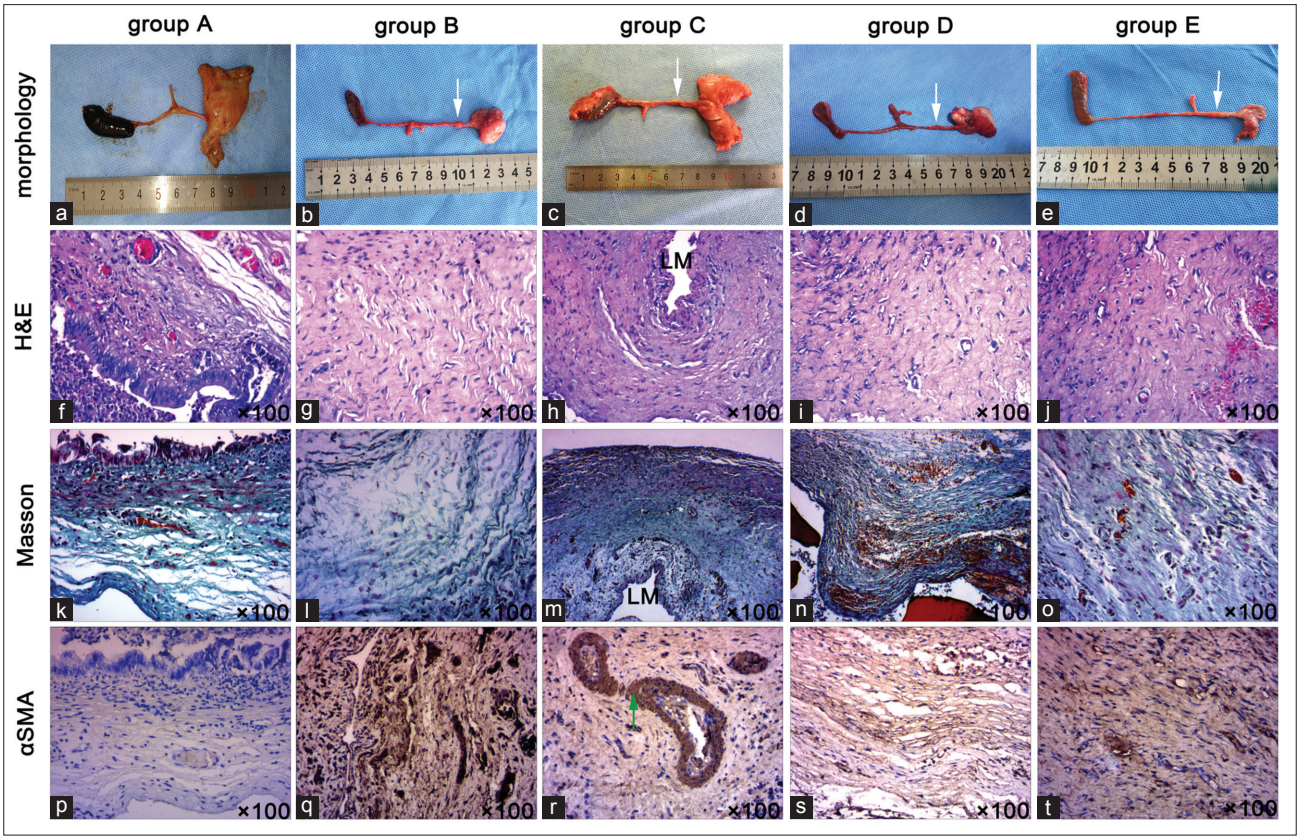

Figure 3: Image of paraffin-embedded tissues in pathology from five groups. (a-e) Images of rabbits' bile duct system at the time of sacrifice, 28 days after implanting. The morphological specimen in the Groups B or C showed the formation of the scar at bile duct away from the superior margin of the duodenum (white arrows) and dilation of common bile duct above scar level; significant morphological changes of bile duct were observed. (f-j) Hematoxylin and eosin staining of the tissue slice of bile duct showed that the thickness of the bile duct in the Groups B or C was much higher than those in Groups A, D, or E (×100). (k-o) Masson's staining of the tissue slice of bile duct from five groups. The collagen was stained with blue, and nuclei to black, cytoplasm, muscle, erythrocytes to red (×100). (p-t) The α-SMA immunohistological staining of the tissue slice of bile duct from five groups. The α-SMA in both myofibroblasts and smooth muscle cells was stained with brown. Green arrow indicated the vascular smooth muscle cells as positive controls (×100). LM: Lumen side of the bile duct; SMA: Smooth muscle actin.

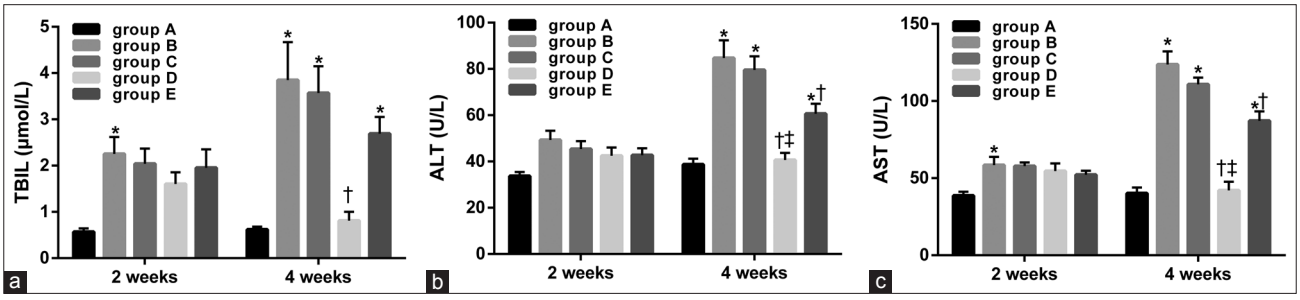

Figure 4: Effect of PTX-SHEC films on live function. Two or four weeks after surgery, the changes in biochemical indexes of rabbits, including TBIL (a), ALT (b), and AST (c). *$P < 0.05$, compared with Group A; †$P < 0.05$, compared with Group B; ‡$P < 0.05$, compared with Group E. AST: Alanine aminotransferase; TBIL: Total bilirubin; ALT: Alkaline phosphatase; SHEC: N-succinyl-hydroxyethyl chitosan; PTX: Paclitaxel.

tract operation by cutting into suitable sizes.[24] To obtain free carboxyl group, SHEC is prepared by HEC and succinic anhydride via ring-opening reactions. Due to the existence of $^{-}NH_2$ and $^{-}COOH$ groups in its structure, SHEC shows good hydrophilicity and can easily react with many kinds of agents.[23,25] It is valuable for the drug carrier to readily prepare its conjugates with various drugs to avoid vexatious complications.[26] Theoretically, a chemical combination could happen between PTX and SHEC in the presence of an appropriate catalyst. Then, the membrane of PTX-SHEC may be prepared using cross-linked modification. An ideal membrane should fulfill the requirements mentioned above and will be a suitable agent for inhibiting fibrosis and reducing scar formation after biliary tract operation. We confirmed that PTX was successfully introduced using NMR and FT-IR method.

We tested the characteristics of PTX-SHEC film using experiments *in vitro* and *in vivo*. The results of experiments

in vitro showed that PTX was slowly and sustainably released for over 4 weeks. In the experiments *in vivo*, the released PTX could be detected in serum concentration after 28 days despite a decrease in amount of PTX released after 7 days. A burst of PTX which may cause local toxicity damage is one of the most general concerns for a delivery system. Dhanikula *et al*.[27] used liposomes or poloxamer 407 to conjugate PTX into CTS film, which released 10% of PTX in only 6 h. The release speed was too high which would cause local toxicity. In this study, PTX-SHEC film which was made of chemical bonds (ester bonds) could slowly and sustainably release 10% of PTX in the first 2 weeks, avoiding a local toxicity of PTX. Drug release experiment *in vitro* showed that the volume of PTX release increased along with the degrees of cross-linking. It might be because the PTX not only bound to SHEC but also was encapsulated in the membranes due to tight cross-linking among the SHEC molecules. This finding supported that the amount of PTX released from the film could be controlled by regulating the degree of cross-linking. Moreover, the results of *in vitro* study supported that the PTX-conjugated SHEC film had no toxic effects in cell viability.

Finally, the results of H&E and Masson's staining showed that PTX-SHEC film could significantly inhibit the formation of PC and reduce duct wall thickness at wound site. However, no inhibiting effect was found in the PTX-SHEC-conjugated film. Our investigation of the α-SMA expression found a significant decrease in PTX-SHEC film-treated rabbits. A recent study showed that PTX-coated stents did not significantly increase the duration of stent patency or survival time when compared with nondrug-eluting covered metal stents.[8] Presumably, this could be due to unsteady release of PTX from the stents for a long enough time or because released PTX was eluted by bile flow. The levels of TBIL, ALT, and AST in PTX-SHEC-treated rabbits remained high at week 2, but dramatically decreased at week 4. The increasing and high levels of elevated liver enzymes at week 2 might be due to the surgery itself which caused local edema and the temporary obstruction of the bile duct, followed by increased bile pressure, leading to hepatocellular rupture. After 4 weeks, biliary edema subsided, PTX-SHEC film prevented scar formation, and liver function recovered. The present study also supported that PTX could be sustained release from PTX-SHEC film for 4 weeks, which was another benefit for rehabilitation of the patients.

Although this study showed promising results, further examination of the film is necessary before clinical application, because of possible occurrence of side effects due to high concentration of PTX. In Lee *et al*.'s study, biliary mucosal hyperplasia was noted in 3/6 dogs which were given with 20% wt/v PTX-incorporated stents.[9] The studies of Lee *et al*.[28] and Jang *et al*.[29] found acceptable histological changes at 28 days after PTX-eluting stent placement, with no incidence of biliary obstruction or perforation. Mild adverse effects were observed in previous study.[30] In this study, no animal died because of toxicity of PTX, and no serious side effect was observed. The limitations herein included a small sample size and a short-observed time. Further evaluation over a long period is warranted.

In conclusion, this study successfully designed and synthesized a CTS membrane conjugated with PTX, allowing long period and low dose of PTX release around the bile duct. The slow and steady release of PTX reduced the chance of benign biliary stricture. Further clinical trial and improvement of the membrane could help make it useful to prevent benign biliary stricture clinically.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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紫杉醇偶联N-琥珀酰-羟乙基壳聚糖膜对兔胆道狭窄模型中增殖性胆管炎的影响

摘要

背景：紫杉醇能抑制增殖性胆管炎中成纤维细胞的生长，其是导致胆管狭窄的重要原因。然而，由于生物利用度较差，局部用药时间过短，紫杉醇临床应用受到限制。本研究设计开发了一种新型紫杉醇缓释膜（紫杉醇偶联的N-琥珀酰-羟乙基壳聚糖膜，PTX-SHEC），并通过体内和体外实验评价其安全性和有效性。

方法：通过核磁共振和傅立叶变换红外光谱测量验证紫杉醇与N-琥珀酰-羟乙基壳聚糖（SHEC）的偶联。使用高效液相色谱法测定体内和体外的药物释放。使用MTT实验测量体外细胞活力。构建胆道狭窄兔模型，并将SHEC或PTX-SHEC缓释膜分别植入模型中（分别定义为B，C，D组；每组8只）。同时将假手术组定义为A组，1000 μmol/L 紫杉醇作为E组（每组8只）。酶联免疫吸附试验检测各组肝功能和残留剂量。苏木素伊红染色和免疫组织化学染色检查胆总管的组织病理学和α-平滑肌肌动蛋白表达情况。

结果：核磁共振和红外光谱表明紫杉醇成功偶联至缓释膜中，其于7.41–7.99 ppm、1.50 ppm和1.03 ppm检测到紫杉醇结构中的质子。动物模型中没有观察到胆汁泄漏。紫杉醇缓释膜可缓慢释放紫杉醇4周（第30天为8.89±0.03 μg）。体外细胞活力测试显示偶联或不偶联紫杉醇膜之间的毒性水平存在显著差异（111.7±4.0％ vs. 68.1±6.0％，P<0.001），而在三组含紫杉醇的膜中未观察到毒性水平存在显著差异（67.7±5.4％，67.2±3.4％和59.1±6.0％，P> 0.05）。组织病理学显示植入28天后，D和E组（非C组），胆道损伤部位的肉芽组织和腺体增生较B组明显减少，其中D组较E组变化更为明显。D组和E组中α-SMA阳性细胞率较少。与E组比较，D组肝脏功能明显改善，比较如下（E vs. D）：总胆红素 (2.69±1.03 μmol/L vs. 0.81±0.54 μmol/L, P=0.014)、谷草转氨酶(87.13±17.51 U/L vs. 42.12±15.76 U/L, P=0.012)、谷丙转氨酶(60.61±12.31 U/L vs. 40.59±8.78 U/L, P<0.001)。

结论：紫杉醇缓释膜可有效抑制胆道重建愈合过程中肌成纤维细胞增殖和细胞外基质过度沉积。这种新型缓释膜为降低良性胆道狭窄的发生提供了新的途径。