Original Article

Paclitaxel Drug-eluting Tracheal Stent Could Reduce Granulation Tissue Formation in a Canine Model

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Abstract

Background: Currently available silicone and metallic stents for tracheal stenosis are associated with many problems. Granulation proliferation is one of the main complications. The present study aimed to evaluate the efficacy of paclitaxel drug-eluting tracheal stent in reducing granulation tissue formation in a canine model, as well as the pharmacokinetic features and safety profiles of the coated drug.

Methods: Eight beagles were randomly divided into a control group (bare-metal stent group, n = 4) and an experimental group (paclitaxel-eluting stent group, n = 4). The observation period was 5 months. One beagle in both groups was sacrificed at the end of the 1st and 3rd months, respectively. The last two beagles in both groups were sacrificed at the end of 5th month. The proliferation of granulation tissue and changes in tracheal mucosa were compared between the two groups. Blood routine and liver and kidney function were monitored to evaluate the safety of the paclitaxel-eluting stent. The elution method and high-performance liquid chromatography were used to characterize the rate of *in vivo* release of paclitaxel from the stent.

Results: Compared with the control group, the proliferation of granulation tissue in the experimental group was significantly reduced. The drug release of paclitaxel-eluting stent was the fastest in the 1st month after implantation (up to 70.9%). Then, the release slowed down gradually. By the 5th month, the release reached up to 98.5%. During the observation period, a high concentration of the drug in the trachea (in the stented and adjacent unstented areas) and lung tissue was not noted, and the blood test showed no side effect.

Conclusions: The paclitaxel-eluting stent could safely reduce the granulation tissue formation after stent implantation *in vivo*, suggesting that the paclitaxel-eluting tracheal stent might be considered for potential use in humans in the future.

Key words: Canine; Drug-eluting Stent; Paclitaxel; Tracheal Stenosis

Introduction

The most common types of stents in clinical use are made of metallic wire or silicone. Currently available silicone and metallic stents for tracheal stenosis are associated with many problems.[1-3] Granulation proliferation is one of the main complications, especially for uncovered metallic stents, which leads to restenosis and affects long-term efficacy.

Studies show that the development of tracheal granulation tissue represents a fibrotic airway repair process that involves fibroblasts[4,5] and it can be partially inhibited using antiproliferative drugs. Paclitaxel has shown significant antiproliferative activity against various cells, such as vascular smooth muscle cells, fibroblasts, endothelial cells, and so on.[6-8] Since paclitaxel-coated stents have been widely used and found to be effective and safe to inhibit restenosis in cardiology, vascular surgery, and other fields,[9-12] it is suggested that the formation and development of tracheal granulation tissue may be partially inhibited using paclitaxel locally in the tracheal mucosa.

Unlike conventional intravenous injection, endobronchial local application of paclitaxel exhibits no significant systemic toxicity. However, such drug delivery is transient and can be prolonged for only a few hours to days in the gel-form or oil-form injection.[13] Hence, the maintenance time after a single administration of paclitaxel cannot meet the

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.193461

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Received: 30-06-2016 **Edited by:** Yuan-Yuan Ji

How to cite this article: Wang T, Zhang J, Wang J, Pei YH, Qiu XJ, Wang YL. Paclitaxel Drug-eluting Tracheal Stent Could Reduce Granulation Tissue Formation in a Canine Model. Chin Med J 2016;129:2708-13.

requirements of inhibiting scarring. If the paclitaxel-eluting tracheal stent can be prepared to achieve the long-term slow release of paclitaxel in the local airway, it can be a solution to this problem.

It has been confirmed that paclitaxel could inhibit primary cultured airway fibroblast proliferation.[8] A paclitaxel drug-eluting stent (DES) with a backbone of poly (lactic-co-glycolic acid) (PLGA) was designed. An *in vitro* experiment showed that paclitaxel drug-eluting tracheal stent could achieve sustained slow release and meet the requirement of drug loading.[14] Following the previous work, this study aimed to observe the efficacy and safety of the paclitaxel drug-eluting tracheal stent *in vivo*.

Methods

Materials

Bare nitinol tracheal stents [40 mm × 16 mm, Figure 1] were provided by the Micro-tech Co., Ltd (Nanjing, China). Paclitaxel (99.6% purity) was purchased from the National Institutes for Food and Drug Control, Beijing, China. PLGA (molecular weight 10,000, ratio of lactic acid/glycolic acid is 75:25) was purchased from the Shandong Medical Equipment Institution in China. Dichloromethane was purchased from the Sinopharm Chemical Reagent Co. (Shanghai, China).

Preparation of paclitaxel-eluting stents

Preparation of paclitaxel DES was in accordance with the requirements of previous studies.[14] The stents were cleaned using an ultrasonic cleaning method with dichloromethane and distilled water in sequence. The cleaned stents were kept under a fume cupboard for 24 h to evaporate the residual water. The stents were then dipped vertically into a coating solution prepared by dissolving 0.45 g (2% w/v) of PLGA and 0.0225 g (0.1% (w/v]) of paclitaxel in 22.5 ml of dichloromethane. The samples were kept at 37°C while being constantly agitated at 75 r/min in an incubator shaker for 24 h. Finally, the paclitaxel-eluting stents were placed in an oven overnight at 37°C to remove any solvent.

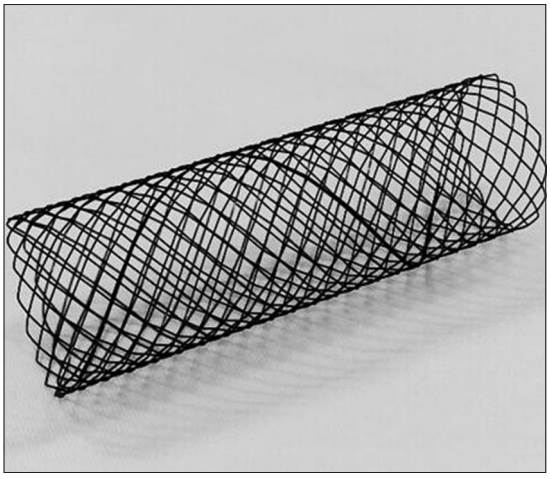

Figure 1: Bare nitinol tracheal stents.

Stent deployment procedure

The canine model is the most commonly used model in airway research because of similar anatomy to that of humans, long accessible trachea, and cooperativeness.[15] All animal procedures were approved by the Institutional Review Board of Beijing Tian Tan Hospital, Capital Medical University (Approval ID: JS2013-007-02). All animals were treated humanely.

Eight beagles (provided by the Academy of Military Medical Sciences, China) with an average weight of 8.1 kg (range 8.0–8.2 kg) were used in this study. Each underwent chest computed tomography scan, and the diameter of the middle trachea was determined according to the following formula: tracheal diameter = (anteroposterior diameter + transverse diameter)/2. The average tracheal diameter was 15.39 ± 0.20 mm (range 15.00–15.60 mm).

The animals were anesthetized with an intramuscular injection of "846 mixture" (0.15 ml/kg; a mixture of ethylenediaminetetraacetic acid, dihydroetorphine, and haloperidol, purchased from the Academy of Military Medical Sciences, China) and an intraperitoneal injection of 3% sodium pentobarbital (0.5 ml/kg). All animals maintained spontaneous breathing in a supine position with intubation.

The simple random sampling method was used, and the beagles were randomly divided into two groups. A total of eight stents were implanted endoscopically in the trachea of animals. Four stents were in control group (bare-metal stent group), and four were in experimental group (paclitaxel-eluting stent group), respectively. The stent was adjusted (if necessary) using forceps to make sure the stent was implanted in the middle of the trachea.

In vivo evaluation of paclitaxel-eluting stents

Each beagle was observed daily for respiratory distress and well-being. Their airways were evaluated weekly using a bronchoscope.

One beagle was sacrificed in both control group and experimental group at the end of the 1st and 3rd months, respectively. In the 5th month, two animals in control group developed obvious respiratory distress, and bronchoscopy showed severe tracheal stenosis. Therefore, the experiment was suspended, and all animals were humanely euthanized.

The trachea (in the stented areas) was excised and cut longitudinally for anatomical observation. The trachea at the lower edge of the stent was cut off and divided into two parts. One part was fixed in a formalin solution for histological examination and the other in a buffered glutaraldehyde solution for the scanning electron microscopic (SEM) study. Blood was collected by syringes through puncture on the forelimb vein for high-performance liquid chromatography (HPLC) analysis. The trachea (in the stented and adjacent unstented areas) and lung tissue from the right upper lobe were excised and submitted for HPLC analysis. The stents were taken out and divided into two

parts, one for SEM study and the other one eluted for the HPLC analysis to evaluate drug release.

Two beagles in experimental group were chosen and their blood was collected by syringes through puncture on the forelimb vein before operation and at postoperative week 1, week 2, month 1, month 3, and month 5 to test blood routine and liver and renal functions.

Results

All animals recovered well after the implantation of these stents. All stents had good expansion, and no migration occurred. Blood test results showed no bone marrow suppression, no obvious liver and kidney dysfunction occurred after stent implantation in experimental group.

Bronchoscopy findings and anatomical findings

The bronchoscopy findings and the anatomical findings 1, 3, and 5 months after stent implantation in control group and experimental group are shown in the Supplementary Figure 1 and the Figure 2.

In control group, a little granulation tissue formed in the 1st month [Supplementary Figure 1a and Figure 2a, black arrow], and then the granulation tissue gradually increased [Supplementary Figure 1b and Figure 2b, white arrow]. In the 5th month, severe granulation tissue could be seen in two beagles [Supplementary Figure 1c and Figure 2c, 2d].

In experimental group, mucosal congestion was found in the 1st month [Supplementary Figure 1d and Figure 2e, black arrow], and then epithelialization around the stent could be seen [Supplementary Figure 1e and Figure 2f, white arrow]. In the 5th month, the area of epithelialization increased [Supplementary Figure 1f and Figure 2g, 2h]. At the upper edge of one paclitaxel DES, some granulation tissue formed [Figure 2h, white arrow], but the middle and lower parts of the stent were smooth. Compared with control group, the formation of tracheal granulation in experimental group was significantly alleviated.

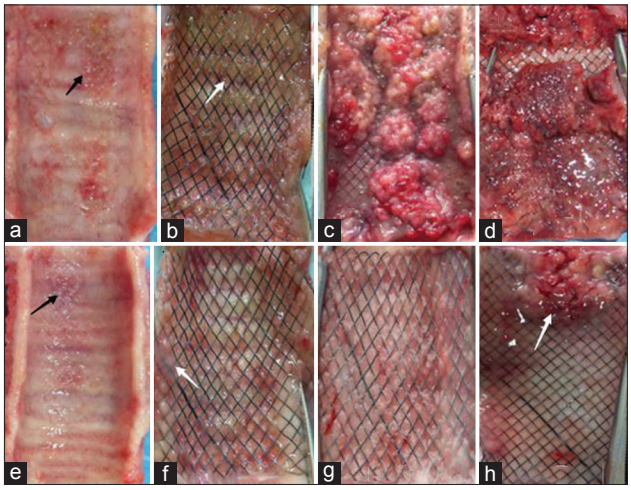

Figure 2: Anatomical findings. (a and b) One and three months after stent implantation in control group; (c and d) 5 months after stent implantation in control group; (e and f) 1 and 3 months after stent implantation in experimental group; (g and h) 5 months after stent implantation in experimental group.

Histological examination for stented trachea

The trachea at the lower edge of the stent was cut off for histological examination. In control group, respiratory epithelial damage with missing cilia on the luminal surface was observed 1 month after stent implantation [Figure 3a, black arrow]. Chronic inflammatory response and missing cilia were noted in the 3rd month [Figure 3b, black arrow]. Five months after stent implantation, the significant proliferation of granulation tissue was observed [Figure 3c, black arrow].

In experimental group, dense infiltrations of inflammatory cells were found in the submucosa in the 1st month after stent implantation [Figure 3d, black arrow], but cilia were present. Three months after stent implantation, epithelial erosion and missing cilia were observed in experimental group [Figure 3e, black arrow]. Five months after stent implantation, respiratory epithelium with normal cilia was formed [Figure 3f, black arrow], and no obvious proliferation of granulation was found.

Scanning electron microscopic examination for luminal surface of stented trachea

The trachea at the lower edge of the stent was cut off for SEM examination. SEM examination showed that in control group, cilia appeared broken and missing on the luminal surface 1 month after stent implantation [Figure 4a]. This phenomenon was more serious in the 3rd month [Figure 4b]. Five months after stent implantation, obvious distortions and missing cilia were found [Figure 4c].

In experimental group, edema was observed 1 month after stent implantation [Figure 4d], and recovery occurred in the 3rd month [Figure 4e]. Five months after stent implantation, the recovery of cilia was close to normal [Figure 4f].

Paclitaxel level in blood and tissue

The paclitaxel levels in the stented trachea were 50.21 mg/kg at postoperative month 1, 16.03 mg/kg at postoperative month 3, and 5.23 mg/kg at the end of the observation

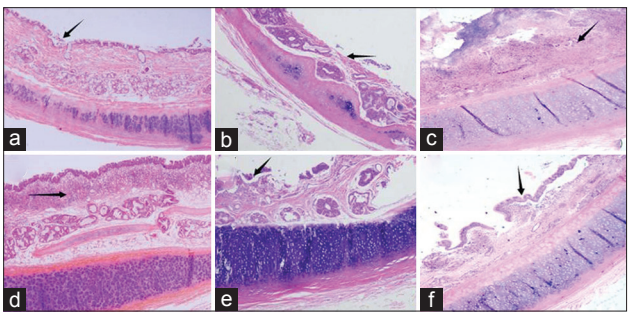

Figure 3: Histological examination of stented trachea (original magnification ×40). (a–c) 1, 3, and 5 months after stent implantation in control group. (d–f) 1, 3, and 5 months after stent implantation in experimental group.

period (5 months). Similar trends were observed in the tracheal segment near the stent (35.42, 12.27, and 3.52 mg/kg, respectively, at postoperative months 1, 3, and 5) and in the right upper lung parenchyma (7.36, 3.27, and 0 mg/kg, respectively, at postoperative months 1, 3, and 5). The serum level of paclitaxel was very low and could not be measured.

Release of paclitaxel from the drug-eluting stent

Scanning electron microscopic examination for the drug-eluting stent

Figure 5 shows the SEM image for bare-metal stent and paclitaxel DES. A smooth, uniform black coating could be seen on the surface of DES. With PLGA degradation, many voids began to appear on the coating surface of DES [Figure 6a, white arrow]. Then, the coated drug degraded further [Figure 6b, white arrow], and almost disappeared at the end of the observation period [Figure 6c, white arrow].

Drug loading on drug-eluting stent

Before implantation, the drug loading on DES was 3161.20 ng. At postoperative months 1, 3, and 5, the drug loading on DES was 921.36, 285.20, and 48.80 ng, respectively. The drug release in the 1st month was the fastest (70.9%). It then gradually slowed down and reached up to 91.0% in the 3rd month. In the 5th month, DES was almost completely released (98.5%).

Discussion

After stent implantation into the tracheobronchial system, granulation formation with subsequent restenosis is one of the main complications, especially for uncovered metallic stents.[16-18] Although patients with malignancy usually have a low-performance status when stents are applied as palliative treatment, a long-term local therapy through airway stents for these patients is welcomed.[19] In some benign disease groups, such as serious tuberculosis, gas exposure, and inhalation burns, the multiplicity of involvement or the long length of stenosis segment increases the surgical challenge. Therefore, endoscopic tracheal stent placement can be an acceptable palliative treatment. Thus, a better long-term prognosis is needed.

It is known that a fibrotic airway repair process involves fibroblasts that cause recurrent, excessive scar formation.[4,5] It can be partially inhibited using antiproliferative drugs. The main focus of the present study was on the degree of reduction of granulation tissue formation by employing a stent with antiproliferative coating (paclitaxel), as well as on the pharmacokinetic features and safety profiles of the coated drug.

The concept of a DES is not new, but most successful examples are in the cardiology field. Only a few experimental studies examining tracheobronchial stents with antiproliferative coating have been published till now. Chao *et al*.[13] developed cisplatin-eluting biodegradable stents, and their experimental results showed that the biodegradable stents released high concentrations of cisplatin *in vitro* and *in vivo* (rabbits) for at least 4 weeks. Zhu *et al*.[20] developed another bioabsorbable tracheal stent with mitomycin C drug elution, and the group of animals with DESs showed less tracheal obstruction and mucus trapping compared with the bare-metal stent group. Sigler *et al*.[21] developed sirolimus-coated stents but found no difference between coated and uncoated stents with regard to the quality and quantity of tissue proliferation.

It is speculated that the difference in the results of various studies might be due to the differing mechanism of action of the antiproliferative coating. The present study found that the paclitaxel-coated stent group showed less tracheal obstruction compared with the bare-metal stent group. At the upper edge of one paclitaxel-coated stent, some granulation tissue appeared. This was also observed in other studies, and autopsy revealed a local injury in the aforementioned location.[22] Therefore, it is presumed that injury to the tracheal mucosa can increase the incidence of granulation tissue proliferation. Therefore, during stent

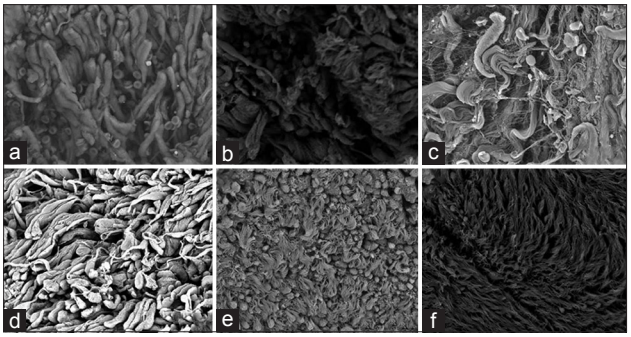

Figure 4: Scanning electron microscopic images for the luminal surface of the stented trachea (original magnification ×2000). (a–c) 1, 3, and 5 months after stent implantation in control group. (d–f) 1, 3, and 5 months after stent implantation in experimental group.

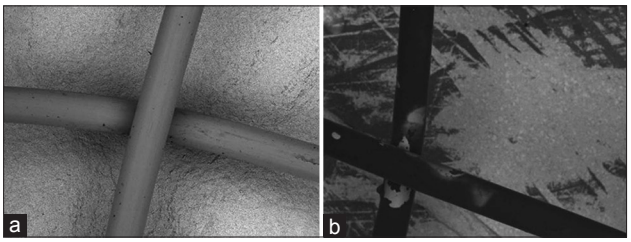

Figure 5: Scanning electron microscopic images (original magnification ×100) for bare-metal stent (a) and paclitaxel drug-eluting stent (b). A smooth, uniform coating could be seen on the surface of drug-eluting stent.

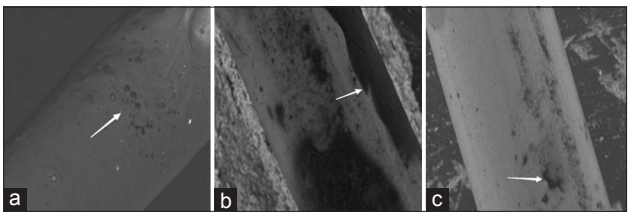

Figure 6: Scanning electron microscopic images of drug-eluting stent (original magnification ×500). Images of drug-eluting stent *in vivo* for 1 month. (a) Many voids appeared on the coating surface (white arrow). Three (b) and five months (c) later, the coated drug almost disappeared (white arrow).

implantation, damage to the normal tracheal mucosa should be avoided.

Compared with the degradation *in vitro,*[14] the present study showed a slower degradation rate *in vivo*. It is speculated that this might be because the water uptake *in vivo* was much less than that *in vitro* and reduced the rate of backbone hydrolysis.[23] The result indicated that the drug release from the paclitaxel-eluting stent had three different stages of release kinetics: an initial burst, a diffusion-controlled release, and a degradation-controlled release. This result was consistent with the findings of other similar studies.[13,24] In addition, the release period could be adjusted by changing the ratio of PLGA/paclitaxel.[13]

The inherent growth-inhibitory properties of many anticancer agents make them ideal candidates for preventing restenosis.[19] However, one of the major concerns is the unpredictable local and systemic toxicity caused by the coated drug. In the present study, a high concentration of paclitaxel was not noted in the trachea (in the stented and adjacent unstented areas) and lung tissue during the observation period, and blood test showed no side effect. The results also showed that the PLGA had good safety and tissue compatibility. They also confirmed that in an appropriate concentration range, paclitaxel did not cause necrosis of the surrounding tissue and did not affect the healing and epithelialization of the tracheal mucosa.[25,26]

The significance of the present results was limited due to the small sample size. However, the results can guide further research on drug-eluting tracheal stents. Moreover, in this study, the drug-eluting model was constructed on nondiseased beagle trachea. The efficacy of stents in humans remains unknown. Therefore, future studies should consider the performance status, comorbidities, extent of disease, and anatomical malformations of patients.

The present study preliminary investigated the efficacy of the paclitaxel drug-eluting tracheal stent in reducing granulation tissue formation in a canine model. The results implied that the paclitaxel-eluting stent could reduce the granulation tissue formation after stent implantation, and it was safe *in vivo*. The potential use of PLGA for sustained release of lipophilic drugs such as paclitaxel was established. The results indicated that the paclitaxel-eluting tracheal stent developed in this study might be considered for potential use in humans in the future.

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

Financial support and sponsorship

This work was supported by a grant of Clinical Technical Innovation Project from Beijing Hospital Authority (No. XMLX201314).

Conflicts of interest

There are no conflicts of interest.

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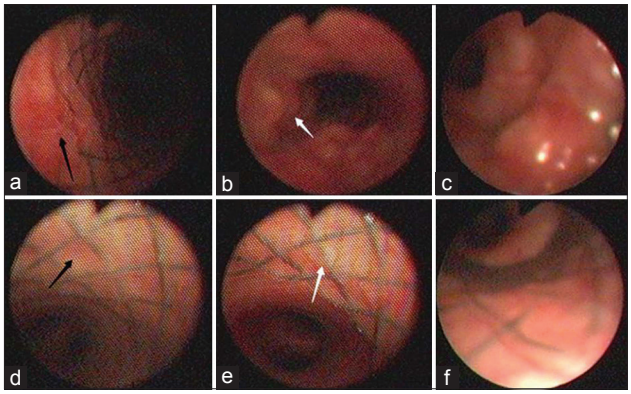

Supplementary Figure 1: Bronchoscopy findings. (a–c) 1, 3, and 5 months after stent implantation in control group. (d–f) 1, 3, and 5 months after stent implantation in experimental group.