Temporary placement of a paclitaxel or rapamycin-eluting stent is effective to reduce stenting induced inflammatory reaction and scaring in benign cardia stricture models

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ABSTRACT

Background/Aims: To investigate whether temporary placement of a paclitaxel or rapamycin eluting stent is more effective to reduce stenting induced inflammatory reaction and scaring than a bared stent in benign cardia stricture models.

Materials and Methods: Eighty dog models of stricture were randomly divided into a control group (CG, n=20, no stent insertion), a bare stent group (BSG, n=20), a paclitaxel eluting (Pacl-ESG, n=20) and a rapamycin eluting stent group (Rapa-ESG, n=20), with one-week stent retention. Lower-oesophageal-sphincter pressure (LOSP), 5-minute barium height (5-mBH) and cardia diameter were assessed before, immediately after the procedure, and regularly for 6 months. Five dogs in each group were euthanized for histological examination at each follow-up assessment.

Results: Stent insertion was well tolerated, with similar migration rates in three groups. At 6 months, LOSP and 5-mBH improved in Pacl-ESG and Rapa-ESG compared to BSG (p<0.05), with no difference between Pacl-ESG and Rapa-ESG (p>0.05). Cardia kept more patency in the Pacl-ESG and Rapa-ESG than in BSG (p<0.05). Reduced peak inflammatory reactions and scarring occurred in the Pacl-ESG and Rapa-ESG compared to BSG (p<0.05), with a similar outcome in the Pacl-ESG and Rapa-ESG (p>0.05).

Conclusion: Paclitaxel or rapamycin-eluting stents insertion led to better outcomes than bare stents in benign cardia stricture models.

Keywords: Benign stricture, cardia, stent, paclitaxel, rapamycin

INTRODUCTION

Benign cardia strictures involve the body of the esophagus and the lower esophageal sphincter (LES) and can cause esophageal obstruction (1,2). Our previous study indicated that fibroblast proliferation and tissue hyperplasia after a temporary stent placement, and subsequent scar formation after stent removal is one of the main factors influencing long-term recurrence rates (3-5).

Effective disruption of the circular muscle fibers in the LES explains the benefits associated with cardia temporary stent insertion (3,6). The tissue repair induced by stent dilation involves an inflammatory reaction mainly mediated by various cell growth factors that induce proliferation and migration of fibroblasts, infiltration of mononuclear cells, plasma cells and lymph cells, and the deposition of extracellular matrix, which has a great similarity to arterial wall repair after stenting (7,8).

Recently, localized drug delivery from drug-eluting stents is accepted as a promising treatment method to prevent restenosis or inflammatory cell proliferation after stenting (9-11). Paclitaxel and rapamycin has been proven to effectively prevent restenosis and the progression of fibrosis in arteries, the urinary and biliary tracts, the cornea and oesophagus by inhibiting the proliferation of smooth muscle cells and epithelial cells (10,11,12-14). Consequently, persistent paclitaxel or rapamycin eluting at the surface of the cardia stent may have the advantage of inhibiting in-stent tissue hyperplasia and inflammatory reactions in the media of the cardia to reduce scar tissue formation and to improve long-term outcome.

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In this experimental study, we created a dog model of benign cardia stricture to test whether placement of the paclitaxel or rapamycin eluting stent is followed by less fibroblast proliferation, tissue hyperplasia and scaring with comparison to those of bare stents.

MATERIALS AND METHODS

Drug-eluting stent preparation
The novel drug-eluting cardia stent consisted of three parts: a bare metal stent, a drug-eluting nano-fiber membrane, and a stent delivery system. The metal stent used (Guangzhou TK Medical Instrument Co, Ltd, Guangzhou, China) was knitted from a 0.25 mm diameter Ni-Ti alloy wire, coated with an anti-erosion layer to prevent gastric acid corrosion. The stent consisted of a self-expanding, cross-linked stainless steel cylindrical mesh body with a 35-mm cydariform and tubaeform at its head and distal ends to prevent stent migration at the gastroesophageal junction. The diameter of the main body of the stent was increased to 30 mm, and the total stent length was 80 mm when fully expanded. A trisected antireflux valve was added at the junction between the stent body and distal end.

The drug-eluting nano-fiber membrane was knitted using electrospinning techniques with mixed paclitaxel (rapamycin)/ Poly ε-caprolactone (PCL) nano-fibers (paclitaxel/rapamycin: PCL=1:50), as previously described (15). The electrospun scaffolds were collected on the surface of the rolling stent and vacuum dried at room temperature for 24 hours. We compressed and deployed each stent using an 8 mm (approximately 24F) delivery system. The entire stent body was radiopaque to facilitate accurate positioning under fluoroscopy (Figure 1).

Benign cardia stricture model
All protocols were approved by the Animal Research Committee of the sixth affiliated people's hospital and were conducted in accordance with the guidelines of the US National Institutes of Health and European Commission guidelines on Animal Care. Eighty beagle dogs of both sexes weighing between 13 and 22 kg had benzyl-dimethyltetradecylammonium chloride (BAC, 4 mmol/L, 12 mL) injected circumferentially into the LES via endoscopy, resulting in experimental benign cardia strictures. Six months after the BAC injection, endoscopy was used to inspect the severity of the stricture before the stent placement. If a gastroscope could not pass through the cardia, we considered the stricture model to be successful. Animals were then randomized into a control group (CG, n=20, no stent insertion), a bare stent group (BSG, n=20, 1-week retention), a paclitaxel stent eluting group (Pacl-ESG, n=20, 1-week retention) and a rapamycin eluting stent group (Rapa-ESG, n=20, 1-week retention).

Stent insertion and removal
Once a 0.035 inch, 260 cm long stiff exchange wire (Terumo, Tokyo, Japan) had been inserted through the mouth and into the stomach under fluoroscopic guidance, the stent delivery system was introduced along the guidewire and passed through the cardia. The stent was then released, based on esophagographic images under fluoroscopic guidance. Following stent expansion, a repeat barium meal examination was performed to confirm the degree of stent expansion and to exclude oesophageal perforation.

When the predetermined stent retention time had been reached, all stents were removed endoscopically. First, we inserted a gastroscope into the stented cardia to assess any stent migration. We then poured 500 to 1000 mL of ice-cold saline solution through the biopsy channel of the gastroscope to reduce bleeding. We used a foreign body forceps to loosen the stent from the surrounding tissue through the biopsy channel and retrieved the stent by contracting its head. We reintroduced the gastroscope to check for bleeding or torn mucosal membranes.
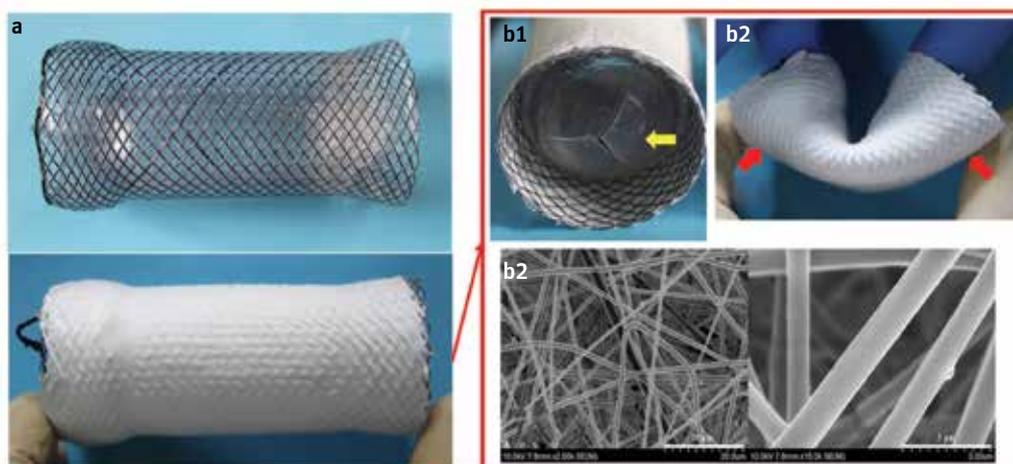
Figure 1. a, b. the lateral view of the fully expanded bare cardia stent **(a)** and a rapamycin-eluting stent **(b)**, with a trisected offset valve designed to prevent reflux a and to allow smooth passage of food **(b-1)**, the fully covered rapamycin-eluting membrane shows good elastic deformation when stent bends **(b-2)**, and a scanning electron microscope image of the nano-fibers of drug-eluting membrane, which ranged from 500-800 nm in diameter **(b-3)**.

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Esophageal manometry and timed barium esophagogram

Lower-oesophageal-sphincter pressure (LOSP) and 5-minute barium height (5-mBH) were assessed under general anesthesia and in the upright position prior to stent placement, and at 1 week, and 1, 3 and 6 months following stent removal, as has been previously described (5,16). Cardia diameter during barium passage in vivo animal models was also measured and compared between groups at each follow-up time-point.

Histological examination

Five animals in each group were euthanized at each follow-up time-point to compare tissue reactions. The resected cardia were fixed in 10% neutral-buffered formalin for a minimum of 48 hours, passed through a series of graded ethanol solutions (70% to 100%) and embedded in paraffin. Serial paraffin-embedded cross sections of the canine cardia were stained with Masson's trichrome to assess collagen proliferation in the submucosal layers (blue, magnification ×400). Cardia samples were also immunostained using mouse anti-proliferating cell nuclear antigen (PCNA) antibody (1:100 dilution; Cell Signaling Technology, Inc. Boston, MA, USA), mouse monoclonal smooth muscle α-actin antibody (1:50 dilution; Abcam Inc., Hong Kong, China) and multiclonal rabbit anti-TGF β1 antibody (1:50 dilution; Santa Cruz Inc. CA, USA) using the Envision immunohistochemical technique. Negative controls were also performed by omitting primary antibodies. The proliferation index was defined as the percentage of PCNA-positive cells against the total number of nucleated cells in the epithelial lining, and the submucosal layer occupied by the α-actin-positive area positive cells. The proliferation index and the percentage of the collagen area in the submucosal layer were calculated using Image-Pro Plus Version 6.0 software (Media Cybernetics, Inc., Bethesda, MD, USA) in at least 20 randomly selected high-power (×400) tubulointerstitial fields from each section. A pathologist who reviewed all the specimens and made the analysis was blinded to the animal randomization, treatment procedure and follow-up protocol.

Statistical analysis

GraphPad Prism 5.0 software (GraphPad Software, Inc; San Diego, CA, USA) and SAS version 9.01 software (SAS Institute, Cary, NC) were used for statistical analysis. A mixed effects linear model statistical analysis was performed to compare overall improvements in LOSP and 5-mBH following stent insertion and at each follow-up time-point to predict outcome. One-way ANOVA was used to compare the PCNA positive cell proliferation index, the smooth muscle α-actin positive area, and the collagen area at each follow-up time-point. Before one-way ANOVA analysis, test for homogeneity of variance and normal distribution of the dependent variables using Shapiro-Wilk test was performed. Fisher's exact test was used to compare stent migration rates between the BSG, Pacl-ESG and Rapa-ESG. Statistical significance was defined as p<0.05.

RESULTS

Animal model and immediate intervention procedure results

Experimental benign cardia stricture was successfully established in all dogs after BAC injection, with the oesophageal manometry and a timed barium esophagram examination increased from 17.70±3.68 mmHg and 1.23±1.09 mm to 55.24±6.11mmHg (p<0.001) and 14.68±1.97 mm (p<0.001). The mean cardia luminal diameters of the dogs decreased from 15.32±4.58 mm before the stricture was formed to 0. 61±0.80 mm afterwards (p<0.001). The BAC injection, and stent insertion and removal were well tolerated and no animal died during the study.
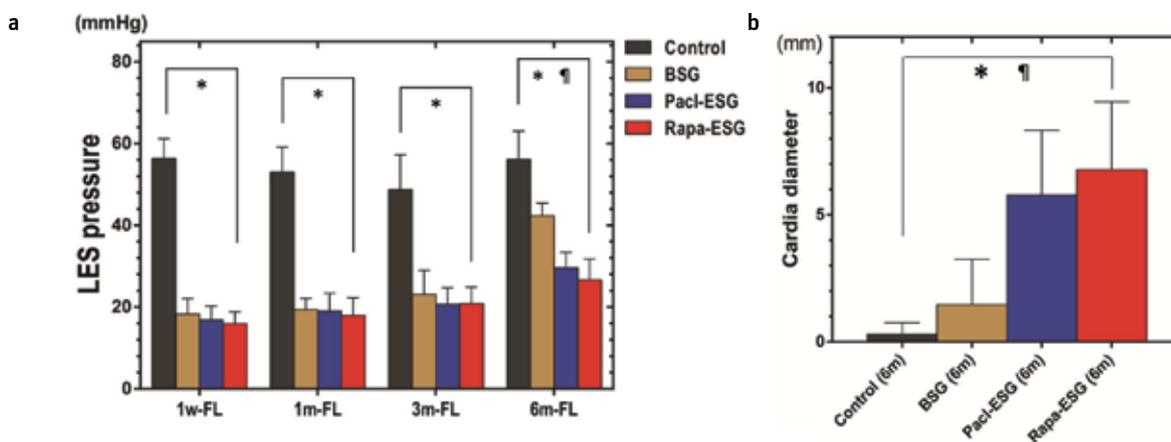
No oesophageal perforations occurred during stent insertion or retrieval. Following stent insertion, any retained barium passed smoothly through the cardia in the BSG, the Pacl-ESG and the Rapa-ESG, compared to slower clearance in the CG. One immediate stent migration into the stomach occurred after stent placement in the Pacl-ESG. When due for removal, stents had migrated into the stomach of one dog in the Rapa-ESG. No migration occurred in the BSG (p=0.3112). Retrieved stents revealed that the biodegradable nano-fiber remained well integrated at 1 week. Procedure-related adverse events, including bleeding during the stent removal procedure and vomiting, were all self-limited.

Oesophageal manometry follow-up

Follow-up oesophageal manometry revealed a gradual increase in LOSP after stent removal in the BSG, Pacl-ESG and Rapa-ESG, more apparent in the BSG, whereas values in Pacl-ESG and Rapa-ESG became more stabilized. At 6 months, the LOSP of the BSG (42.34±3.11 mmHg vs. 56.18±6.89 mmHg in control, P<0.05) was significantly higher than that of Pacl-ESG and Rapa-ESG (p<0.05). The LOSP of Pacl-ESG and Rapa-ESG revealed similar oesophageal manometry remission at 6 months (29.60±3.82 mmHg vs. 26.70±5.02 mmHg, p>0.05) (Figure 2).

Timed barium esophagram follow-up

During oesophageal manometry follow-up, it revealed a gradual aggravation of barium clearance remission after stent removal in the BSG, Pacl-ESG and Rapa-ESG, whereas values in the BSG are more apparent than that in the Pacl-ESG and Rapa-ESG. At 6 months, the 5-mBH of the BSG (11.1±5.46 cm) was significantly higher than Pacl-ESG (6.00±4.72 cm) and Rapa-ESG (6.50±2.98 cm) (p<0.05). Barium clearance of Pacl-ESG was similar to that of Rapa-ESG at 6 months (p>0.05). (Figure 3) After different interventions, the cardia diameters in the BSG, Pacl-ESG and Rapa-ESG present better patency than control during the follow-up intervals (p>0.05). At the end of 6-month follow-up, mean cardia diameters in Pacl-ESG and Rapa-ESG were 5.78±2.55 mm and 6.78±2.67 mm, respectively, which were greater than those observed in the BSG (1.46±1.78 mm; p<0.05) (Figure 2).

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Figure 2. a, b. LOSP at regular follow-up intervals in the CG, BSG, Pacl-ESG and Rapa-ESG. At 6 months, the LOSP of the BSG is significantly higher than in Pacl-ESG and Rapa-ESG (p<0.05). The LOSP of Pacl-ESG is similar to that in Rapa-ESG (p>0.05) **(a)**. Caidia diameter measurement at 6 months indicate well patency of the dilated cardia in the Pacl-ESG and Rapa-ESG than that in the BSG (p<0.05). *p<0.05 for comparisons of CG, BSG, Pacl-ESG and Rapa-ESG; ¶p<0.05 for comparisons of BSG, Pacl-ESG and Rapa-ESG **(b)**.
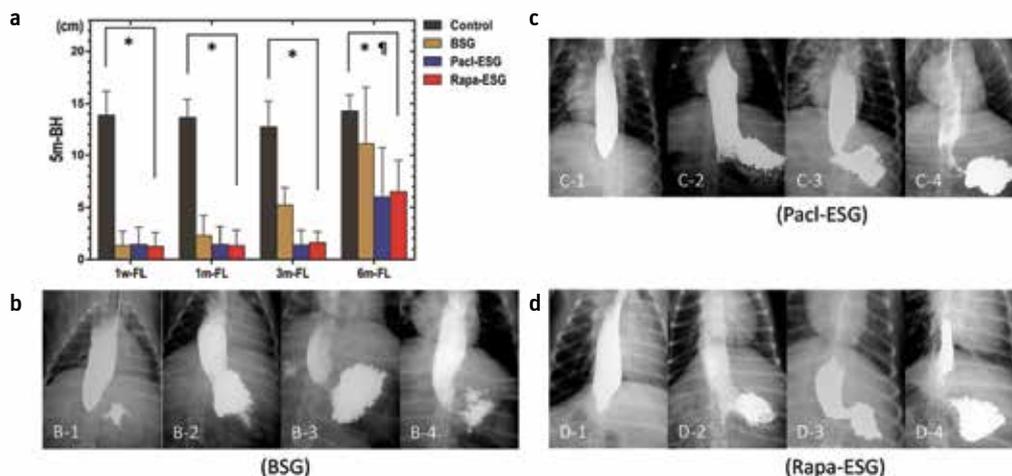
Figure 3. a-d. Oesophageal barium emptying in the CG **(a)**, BSG **(b)**, Pacl-ESG (c) and Rapa-ESG **(d)**. The 5-mBH of benign cardia stricture models in all groups simulated the radiographic findings in clinical patients **(b-1, c-1, d-1)**. After stent deployment, the tight cardia is dilated by the bare stent **(b-2)** and a paclitaxel eluting **(c-2)** or rapamycin eluting **(d-2)** stent. Barium esophagrams reveal good barium clearance at the one-week follow-up assessment in the BSG **(b-3)**, Pacl-ESG **(c-3)** and Rapa-ESG **(d-3)**. At six months, barium emptying clearance at 5 min is rapid in the Pacl-ESG **(c-4)** and Rapa-ESG (d-4), poor in the BSG **(b-4)**. *P<0.05 for comparison of the CG, BSG, Pacl-ESG and Rapa-ESG; ¶P<0.05 for comparisons of the BSG, Pacl-ESG and Rapa-ESG.

Histological examination

As shown in Figure 4, PCNA-positive cells (mainly squamous epithelial cells), and α-smooth muscle actin-positive cells were detected in the epithelial lamina and submucosa. The PCNA and α-actin positive index peaked at 1 month in the BSG, Pacl-ESG and Rapa-ESG. Quantitative analysis of the PCNA and α-actin positive cell proliferation index at 1 month revealed much higher levels in the BSG (71.52±6.00% and 4.86±0.59%, respectively) than in Pacl-ESG (57.16±5.83% and 3.58±0.46%, P<0.05) and Rapa-ESG (57.62±7.09% and 3.46±0.34%, p<0.05). The number of PCNA and α-actin positive cells in Pacl-ESG was similar to that in the Rapa-ESG (p>0.05). Masson›s trichrome staining revealed a gradual increase in collagen fiber proliferation during follow-up after stent treatment, which stabilized at approximately 3 months. At 3 and 6 months, the area of collagen in the BSG (44.32±3.36% and 46.04±3.32%,

respectively) was significantly higher than in the Pacl-ESG and Rapa-ESG (P<0.05), with a similar area in Pacl-ESG and Rapa-ESG (37.12±1.92% and 38.44±3.86% vs. 35.90±3.21% and 38.60±5.25%, respectively; p>0.05) (Figure 4).

DISCUSSION

The main findings were as follows: (1) the success rate for drug-eluting stents placement was 100% in this experimental stricture model, with slightly higher migration rates than observed with bare stents (0% vs. 5%; p=0.3112); (2) at 6 months, dogs who had undergone drug-eluting stent insertion led to better barium clearance and LOSP remission than the bare stent due to reduced inflammatory reactions and scarring induced by stent placement; and (3) paclitaxel and rapamycin eluting stent retention had similar treatment outcomes based barium clearance and LOSP measurement and histological observations.

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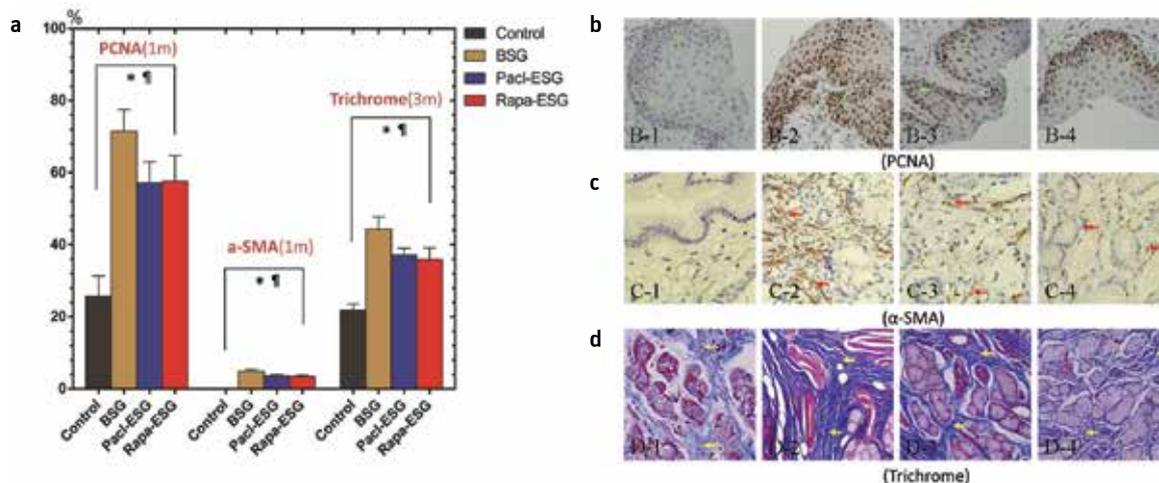
Figure 4. a-d. (a) Pathological changes in the CG, BSG, Pacl-ESG and Rapa-ESG at different follow-up periods. Immunohistochemical staining for PCNA was observed in the cubicula layer (brown, magnification ×400; green arrows, **(b)** and α-SMA staining was observed in the submucosa (brown, magnification ×400; red arrows, **(c)**. The number of PCNA positive proliferating cells and α-SMA positive cells peaked one month after treatment. The number of PCNA and α-SMA positive proliferating cells in the BSG **(b-2, c-2)** was significantly higher than the Pacl-ESG **(b-3, c-3)** and Rapa-ESG **(b-4, c-4)** at 1-month follow-up **(a)**. Masson's trichrome staining indicating that collagen fibre proliferation in the submucosa (blue, magnification ×400; yellow arrows, **(c)** stabilized around three months after treatment. Collagen fibre proliferation in the BSG **(d-2)** was significantly more severe than the Pacl-ESG **(d-3)** and Rapa-ESG **(d-4)** at 3 months. *$P<0.05$ for comparison of the CG, BSG, Pacl-ESG and Rapa-ESG; ¶$P<0.05$ for comparison of the BSG, Pacl-ESG and Rapa-ESG.

Several studies have investigated the pathological mechanisms of arterial stenosis in recent years (17-19). Currently, most of the drugs used in eluting stents aim to inhibit the inflammatory reaction, or reduce cell proliferation and fibrosis to prevent lumen stenosis (11,12). Paclitaxel could stabilize microtubules and as a result, interferes with the normal breakdown of microtubules during cell division to inhibit cell proliferation (11,20). Rapamycin is an immunosuppressive and antiproliferative agent that inhibits the activity of the protein kinase mTOR, a central regulator of mammalian cell growth and proliferation, by blocking activation of signal transduction pathways, causing arrest of the cell cycle in the G1 phase (11,21). Recently, paclitaxel or rapamycin-coated eluting-stents have been used in human coronary arteries to prevent neointima formation, the leading cause of restenosis via inhibiting migration and proliferation of the smooth muscle cells in the media (14,19). The oesophagus tissue repair procedure after temporary stent placement was also a kind of inflammatory reaction mainly mediated by various cell growth factors (like vascular endothelial growth factor and transforming growth factor, type beta) to induce proliferation and migration of fib hjroblasts, infiltration of mononuclear cells, plasma cells and lymph cells, and the deposition of extracellular matrix (7,8). Thus, drug-eluting techniques may be applied to cardia stents, prolonging the stenting period, reducing scar tissue formation and improving long-term outcome.

In our study, the dog model received an injection of the neurotoxin, BAC, to induce a benign stricture of the cardia that was similar to achalasia. BAC has been widely used to establish a model of intestinal deganglionation in many animals, as a result of its ability to injure gastrointestinal neurons (charac-terized by loss of myenteric neurons and increased cholinergic nerve bundles) and the membranes of SMCs membrane in a nonspecific manner. The residual SMCs then become hyperplastic to repair the defect, but neurons cannot regenerate (16,22,23). After the injection of BAC, the denervated LES became persistent, which caused progressive distal esophageal obstruction and esophageal body dilation. These are characteristics of human achalasia that are found on timed barium esophagography.

In this study, we compared outcome and tissue reaction between Pacl-ESG or Rapa-ESG and bare stents at the 1-week stent retention time. At 6 months, enhanced subjective improvement was observed in the Pacl-ESG and Rapa-ESG compared to the BSG for LOSP (29.60±3.82 mmHg and 26.70±5.02 mmHg vs. 42.34±3.11 mmHg; $p<0.05$), 5m-BH (6.00±4.72 cm and 6.50±2.98 cm vs. 11.1±5.46 cm) and carida diameter (5.78±2.55 mm and 6.78±2.67 mm vs. 1.46±1.78 mm; $p<0.05$), suggesting that after 1-week stent dilation, paclitaxel or rapamycin-eluting stents improved cardia restenosis more than bare stents. Consistent paclitaxel or rapamycin elution at the stent surface for 1 week effectively inhibits fibroblast proliferation and migration in the submucosal layer, as demonstrated by α-actin staining (3.58±0.46 % and 3.46±0.34 % vs. 4.86±0.59%; $p<0.05$) at 1 month. There was also less collagen deposition and scar formation in Pacl-ESG or Rapa-ESG than in the BSG at 3 months (37.12±1.92% and 35.90±3.21% vs. 44.32±3.36%; $p<0.05$). The PCNA-positive index revealed that paclitaxel or rapamycin-elution for 1 week more effectively inhibited epithelial cell hyperplasia than bare stents (57.16±5.83% and 57.62±7.09% vs. 71.52±6.00%; $p<0.05$) at 1 month, suggesting less in-stent tissue hyperplasia with drug-eluting stents.

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Outcome comparison and tissue reaction between Pacl-ESG and Rapa-ESG was also performed. At 6 months, paclitaxel or rapamycin elution was associated with similar LOSP (29.60±3.82 mmHg vs. 26.70±5.02 mmHg; p>0.05) and 5m-BH (6.00±4.72 cm vs. 6.50±2.98 cm) remission and carida diameter improvement (5.78±2.55 mm vs. 6.78±2.67 mm; p>0.05). Consistent paclitaxel or rapamycin elution at the stent surface for 1 week could achieve similar fibroblast proliferation inhibition in the submucosal layer, as demonstrated by α-actin staining (3.58±0.46 % vs. 3.46±0.34 %; p>0.05) at 1 month, and similar collagen deposition and scar formation (37.12±1.92% vs. 35.90±3.21%; p>0.05) at 6 months. The epithelial cell hyperplasia in the paclitaxel or rapamycin eluting stents as demonstrated by PCNA staining (57.16±5.83% vs. 57.62±7.09%; p>0.05) also revealed similar tissue hyperplasia inhibition at 1 month after different drug eluting.

It had been proved that our novel paclitaxel- or rapamycin-eluting stent placement was effective to reduce subsequent fibroblast proliferation and tissue hyperplasia after stent placement than bare stents in an experimental stricture dog model after BAC injection. Although these preliminary results are encouraging under experimental conditions, clinical studies are required to confirm its efficacy.

Conflict of Interest: No conflict of interest was declared by the authors.

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