Inhibition of granulation hyperplasia and scar tissue formation by rapamycin-eluting stents in benign airway stenosis: an animal study

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Research Article

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Abstract

Objective To study the inhibitory effect of rapamycin-eluting stents on the proliferation of granulation tissue and formation of scar tissue in a canine model with benign tracheal stenosis.

Methods A benign airway stenosis model of Beagle dogs was created by compressing the trachea with endotracheal intubation and a high-pressure balloon. Six Beagle dogs with comparable degrees of tracheal stenosis were randomly divided into two groups: the rapamycin-eluting stent (RES) group and bare metal stent (BMS) group. Before stent insertion, all the tracheal stenosis were evaluated carefully and dilated with a tracheal balloon. Then, rapamycin-eluting stents and bare metal stents were inserted in the stenosis of Beagle dogs in the RES group and BMS group, respectively. Endoscopic evaluation was performed every 2 weeks after the operation. The degree of inflammation in the airway mucosa and granulation tissue formation in and at both ends of the stents were recorded. After a 12-week observation period, all the Beagle dogs were euthanized, and the tracheas were dissected. Further microscopic evaluation of granulation tissue proliferation and scar tissue formation was undertaken.

Results The scores of mucosal airway inflammation in the RES group were lower than those in the BMS group. The rate of tracheal stenosis caused by granulation tissue proliferation and scar tissue formation in the RES group was significantly decreased in comparison with the BMS group, during the follow-up period. All the Beagle dogs were euthanized after 12 weeks and the tracheal tissue around the stents was dissected for fixation and hematoxylin-eosin (H&E) staining. The degree of granulation tissue proliferation and scar tissue formation in the RES group was significantly lower than that in the BMS group under the microscope.

Conclusions Rapamycin-eluting stents may reduce local airway mucosal inflammation and inhibit granulation tissue proliferation and scar tissue formation in dogs with benign airway stenosis and do so to a higher degree than bare metal stents.

1. Introduction

Benign central airway obstruction is a medical emergency often encountered in clinical practice, as well as airway stenosis. Furthermore, post-intubation and post-tracheotomy tracheal stenosis are the most common causes of acquired benign airway stenosis [1]. Airway stent insertion can rapidly restore the patency of the airway and improve the patient's dyspnea. Nevertheless, there are also complications from airway stent placement, including impaired mucociliary clearance, increased risk of bacterial infection, proliferation of granulation tissue, stent migration and stent fracture [2–4]. Among these complications, the proliferation of granulation tissue is a common occurrence that can lead to airway restenosis and make the removal of the stent difficult. In the past decades, silicon stents, covered stents and biodegradable stents have been developed in order to reduce their associated complications. Although each stent has its own advantages, none can solve the problem of airway restenosis caused by granulation tissue proliferation and scar tissue formation.

In our previous study, we showed paclitaxel-eluting tracheal stents can inhibit granulation tissue proliferation and scar tissue formation due to stent insertion in a canine model [5]. However, this finding has not been yet translated to clinical practice.

Rapamycin, a new immunosuppressant drug, is widely used in coronary drug-eluting stents to prevent coronary restenosis. Clinical studies have showed favorable results of rapamycin-eluting stents when compared to bare stents in the prevention of coronary restenosis [6–8]. Recently, it has been suggested that rapamycin-eluting stents could slow the process of granulation tissue proliferation and scar tissue formation [9].

In the present study, we made a canine tracheal stenosis model induced by compression of the tracheal wall after intubation, with a high pressure endotracheal cuff. We aimed at finding out whether rapamycin drug-eluting stents could reduce the granulation tissue and scar tissue formation in the canine tracheal stenosis model, and compare their effect to that of bare metal stents.

2. Materials And Methods

2.1. Materials and related instruments

Tracheal stents: bare nickel titanium (nitinol) tracheal stents (Weiqiang Medical Technology Co., Ltd (Hangzhou, China, Fig. 1) were used in this study.

Animals: Six adult Beagle dogs of either sex (weight, 9–11 kg) were used in this study (Beijing Keyu Laboratory Animal Science and Technology Co., Ltd.).

Other materials and instruments used were: a. incisive CT (64 rows, Philips, the Netherlands) was used to measure the trachea diameter of the Beagle dogs; b. a rigid tracheoscopy (Karl Storze, Germany) was used to see the stent and airway mucosa and c. Fiber bronchoscope (QG-3490, Zhuhai Shixin, China) was used for airway stent implantation.

2.2. Research Methods

2.2.1. Preparation of rapamycin drug-eluting stents

Bare nitinol tracheal stents were used as the basic structural medium for rapamycin. The steps of preparation of rapamycin RES were as follows: a. the stents were cleaned with a 15 min cycle of ultrasonic oscillation using acetonitrile, absolute ethanol, and deionized water and then dried on standby; b. a mixed solution of polylactic acid glycolic acid copolymer (PLGA) and rapamycin (1:1) was prepared; c. the stents were immersed in the solution mentioned above via dip coating, and pulled out at a constant speed. They were then dried in a vacuum drying chamber at room temperature until the solvent completely evaporated.

2.2.2. Animal Model of benign Tracheal Stenosis

Chest CT scans used for measurement of the tracheal diameter of the Beagle dogs were conducted before the experiment, and tracheal diameter was between 13.6 and 15.5 mm. We referred to the method of making the Beagle dog airway stenosis model by Su Zhuquan [10], which used endotracheal intubation and a high-pressure cuff to compress the tracheal mucosa. The experimental dogs were intubated with an I.D.8.0 endotracheal cuff under general anesthesia, and gas was injected into the cuff to maintain the cuff pressure at 200 mmHg for 24 hours. Repeated intubation procedures were carried out if there was no stenosis in the trachea seven days later. The average time for completion of the animal models was 41.0 ± 11.6 hours. After an observation period of 3 weeks, the rate range of tracheal stenosis was 36.6–59.8%, and the length of tracheal stenosis was between 1.8 and 3.0 cm (Fig. 2). Complete random designing methodology was used to divide six Beagle dogs into rapamycin -eluting stent (RES) group (a-c) and bare metal stent (BMS) group (d-f), with three dogs in each group. There were no significant differences between the RES group and

the BMS group in age, body weight and degree of airway stenosis and length of airway

stenosis (Table 1).

Table 1
The comparison of baseline data between the experimental group (RES) and control group (BMS)

Group	Number	Age(m)	Weight(kg)	Tracheal diameter (mm)	Rate of stenosis (%)	Length of stenosis (cm)	Diameter of stent (mm)	Type of stent
RES group	a	13	12.3	13.7	50.7	1.9	14	RES
	b	12	13.8	13.5	56.0	2.5	14	RES
	c	13	12.6	13.7	40.2	2.3	14	RES
BMS group	d	12	13.1	15.6	49.6	3.0	16	BMS
	e	12	14.0	13.5	63.4	2.2	14	BMS
	f	13	12.5	15.4	46.1	1.8	16	BMS

2.2.3. Stent deployment procedure

The animals were anesthetized with an intramuscular injection of silazine hydrochloride (0.15 mL/kg) and an intraperitoneal injection of 3% sodium pentobarbital (0.5 mL/kg). After successful anesthesia, the animals kept spontaneous breathing and were fixed on the operating table in a supine position. A rigid bronchoscope was employed to see the stenosis and insert the tracheal stent. When necessary, the stent was adjusted using forceps to make sure it would cover the upper and lower edges of stenosis by at least 1 cm.

2.2.4. Postoperative observation

The mental status, appetite and respiratory symptoms (dyspnea, coughing and hemoptysis) of the animals were recorded every day. The location of the stent, degree of airway inflammation and degree of tracheal stenosis caused by the proliferation of granulation tissue were recorded under bronchoscopy every two weeks.

The scoring criteria used for degree of airway mucosal inflammation under bronchoscopy were the following: mild congestion and edema of mucosa at both ends of the stent, with a small amount of attached white secretion(1 point); mildly eroded mucosa and white secretions attached to the mucosa < 3 mm from the end of the stent, with edges of the stent visible (2 points); mucosal erosion at both ends of the stent with white secretions attached to or covering the edge of the stent > 3 mm away from the ends of the stent, or dry gravel like change of the mucosa (3 points).

The airway stenosis rate was defined by the rate of airway stenosis caused by granulation tissue proliferation and scar tissue formation under bronchoscopy (measured by Image J software, National Institutes of Health, Bethesda, MD, USA). The calculation formula used was: (1-area at the highest degree of stenosis/area before stenosis) × 100%.

Blood routine and biochemical indexes were drawn before the operation, at 4 weeks and 12 weeks after the operation. All animals were euthanized and the trachea around the stent segment was obtained for dehydration, fixation, and hematoxylin-eosin (H&E) staining. The degree of granulation tissue proliferation between the two groups was measured under the microscope.

3. Results

Animals in both groups had mild cough and loss of appetite after airway stent insertion. These symptoms subsided in the course of one week. Animals in the BMS group had cough and dyspnea after activity up to 8 to 10 weeks after airway stent insertion, while the animals in the RES group had only mild cough. Blood test results were negative for bone marrow suppression, and liver and kidney functions were normal in both groups 4 and 12 weeks after stent implantation.

3.1. Bronchoscopy findings

The degree of airway mucosal inflammation was observed with bronchoscopy. The inflammatory reaction on the airway mucosa (including mucosal congestion, edema, hemorrhage, and adhesion of airway secretions) was seen around the stent after the stent insertion. The scores of mucosal inflammation in the RES group were lower than those in the BMS group (Fig. 3).

The airway mucosa around the stent presented obvious edema on the second week after stent insertion in the two groups, and a small amount of granulation tissue hyperplasia could be seen around the stent in both groups. At the fourth week, the mucosal edema was reduced but obvious granulation tissue proliferation could be seen under the bronchoscopy in the BMS group. Compared with the BMS group, there was much less granulation tissue proliferation in the RES group. With the gradual worsening of

airway stenosis, animals showed increasing exerted dyspnea and worsening cough in the BMS group. In the RES group, the animals had only slight cough. The airway stenosis rate between the two groups is shown in Fig. 4. On the 12th week, the airway stenosis rates were 15%, 13% and 18% in the RES group and 30%, 95% and 51% in the BMS group.

3.2. Anatomical and histological findings

All the Beagle dogs were euthanized by intravenous injection of excessive phenobarbital sodium on the 12th week. The tracheal section containing the stent was excised and cut longitudinally for anatomical observation. In the RES group, a small amount of granulation tissue could be seen, in the tracheal membrane. A much larger amount of granulation tissue was seen in the BMS group.

3.3. Microscopic examination

The trachea around the stent was dissected and fixed with formalin. The specimens were embedded in paraffin and stained with H&E stain. Under the microscope, there was extensive lymphocytic infiltration of the mucosa, partial cartilage destruction and a small proliferation of collagen fibers around the stents in both groups. The tracheal epithelium and mucosa in the RES group were more uniform and most of the stent was covered by the tracheal mucosa. The proliferation of granulation tissue in the RES group was significantly lower than that in BMS group (Fig. 5).

4. Discussion

Airway stent insertion can quickly and effectively alleviate airway stenosis and can be used as preoperative transition or palliative treatment for patients with severe airway stenosis. Airway restenosis caused by granulation tissue proliferation after stent insertion is the main complication of bare metal stents [11, 12]. Therefore, airway metal stents, especially bare stents, are mostly used for malignant airway stenosis. For benign airway stenosis, metal bare stent insertion is considered only when there is no other alternative choice [13]. However, metal stents also have many advantages when compared with silicone stents, such bigger ease of insertion with electronic bronchoscope under local anesthesia, larger lumen diameter, less migration, and unaffected ciliary removal function [14–16]. Metal stents continue to evolve in terms of drug elution and structure, with reduced complications.. In this study, rapamycin eluting airway stents were inserted into the Beagle dog tracheal model of airway stenosis, and we concluded that rapamycin-eluting stents could reduce local airway inflammation and inhibit the proliferation of granulation tissue, when compared with bare metal stents.

In earlier studies on airway stents, most of the experimental animal models were of normal airways or of airway stenosis models made by argon plasma burning and coagulation of the airway mucosa. Part of the tracheal cartilage and damaged airway mucosa was then removed by brush. However, in clinical practice, benign airway stenosis is mostly caused by endotracheal intubation or tracheotomy [17], which is different from these models. Therefore, in order to replicate this clinical scenario to the greatest extent, we referred to the method of making the Beagle dog airway stenosis model by Su Zhuquan [10], which

uses endotracheal intubation and high-pressure cuff to compress the tracheal wall mucosa. This results in local mucosal and cartilage ischemia and necrosis, and repair with granulation and scar tissue that creates airway stenosis. The average modeling time of this experiment was 40.0 ± 12.4 hours, and all tracheal stenosis rates ranged between 40.2% and 63.4%.

Rapamycin is a new immunosuppressant drug that blocks signal transduction through different cytokine receptors. It blocks transition from stage G_1 to S in T lymphocytes and other cells, and has intensive anti-proliferation and immunosuppressive effects. In mammals, rapamycin acts on mTOR [18], which regulates a variety of cells involved in immune response, such as dendritic cells and regulatory T cells (Tregs). In recent years, rapamycin has been increasingly used in settings of immune inflammation. In asthma models, intraperitoneal injection of rapamycin can reduce the concentration of IL-5 in alveolar lavage fluid and inhibit the aggregation of eosinophils, so as to alleviate the symptoms of asthma [19]. In this study, it was reported that the lung inflammation and the Th2 inflammatory factors IL-4, IL-5 and IL-13 in alveolar lavage fluid and nasal lavage fluid decreased significantly after aerosol inhalation of rapamycin. In this study, the degree of tracheal inflammation in the RES group was significantly lower than that in the BMS group, which further proves that rapamycin can locally inhibit airway inflammation.

Rapamycin eluting drug stents have been proven effective in preventing restenosis after coronary stenting [5–7]. At present, there are few studies on rapamycin eluting airway stents. Madhavi Duvvuri [20] applied rapamycin coated absorbable PLLA-PCL stents to the mouse model of tracheal stenosis. After a period of 6 weeks' observation, rapamycin coated PLLA-PCL stents significantly reduced the thickness of the lamina propria mucosa around the stents and reduced the amounts of collagen 1, 3 and TGF-β, which suggests that rapamycin eluting stents can reduce granulation tissue proliferation and scar tissue formation around the stent. In our study, the degree of granulation tissue proliferation in the RES group was significantly lower than that in the BMS group and at the end of the 12th week, the airway stenosis rates in the RES group were 15%, 13% and 18% while those in the BMS group were 30%, 95% and 51%, respectively. Under the microscope, the degree of granulation hyperplasia in the RES group was significantly less than that in BMS group.

5. Conclusions

In this study, rapamycin eluting airway stents were shown to reduce local airway inflammation and granulation tissue proliferation in animal models with benign airway stenosis, which provides a solid theoretical basis for further research on this topic.

Declarations

Institutional Review Board Statement:

The study was conducted according to the ARRIVE guidelines, and approved by the Institutional Review Board of Beijing Tian Tan Hospital (protocol code: 202101016 and date of approval: 2021.2.2).

Consent for publication

Not applicable

Availability of data and materials

The datasets generated during the current study are not publicly available due to patent application for the drug-eluting stent but are available from the corresponding author on reasonable request.

Conflicts of Interest:

The authors declare no conflict of interest.

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Authors' contributions

Feng Chen and Ting Wang carried out the animal experiment, Xiao-jian Qiu and Jie Zhang supervised the experiment implementation process. All authors read and approved the final manuscript.

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Figures

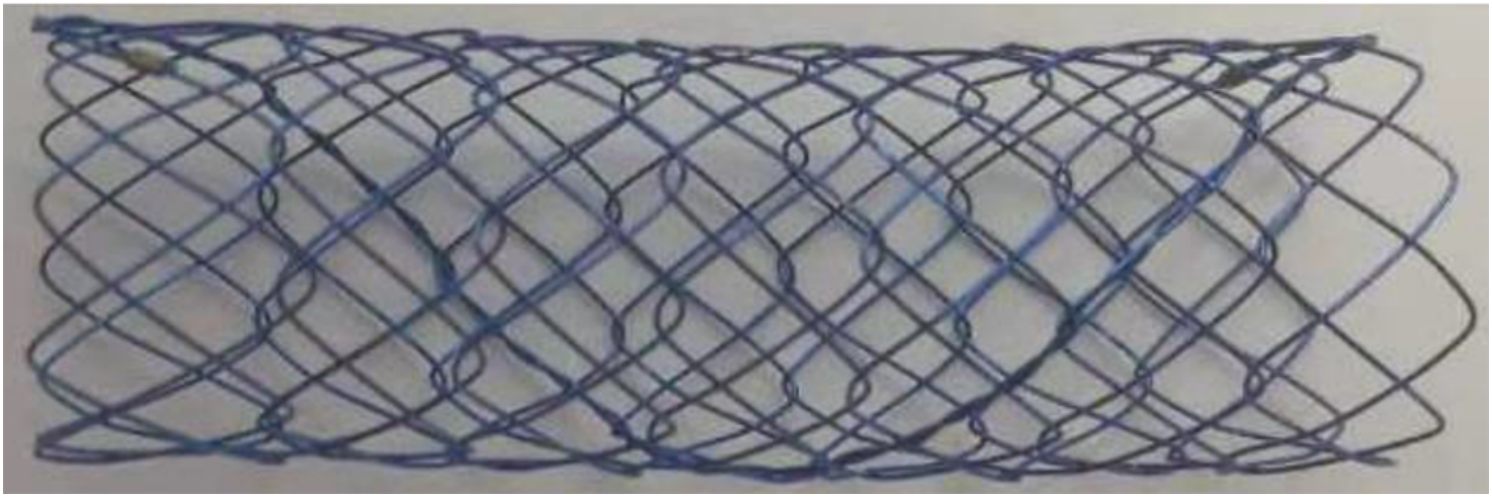

Figure 1

Bare nitinol tracheal stent.

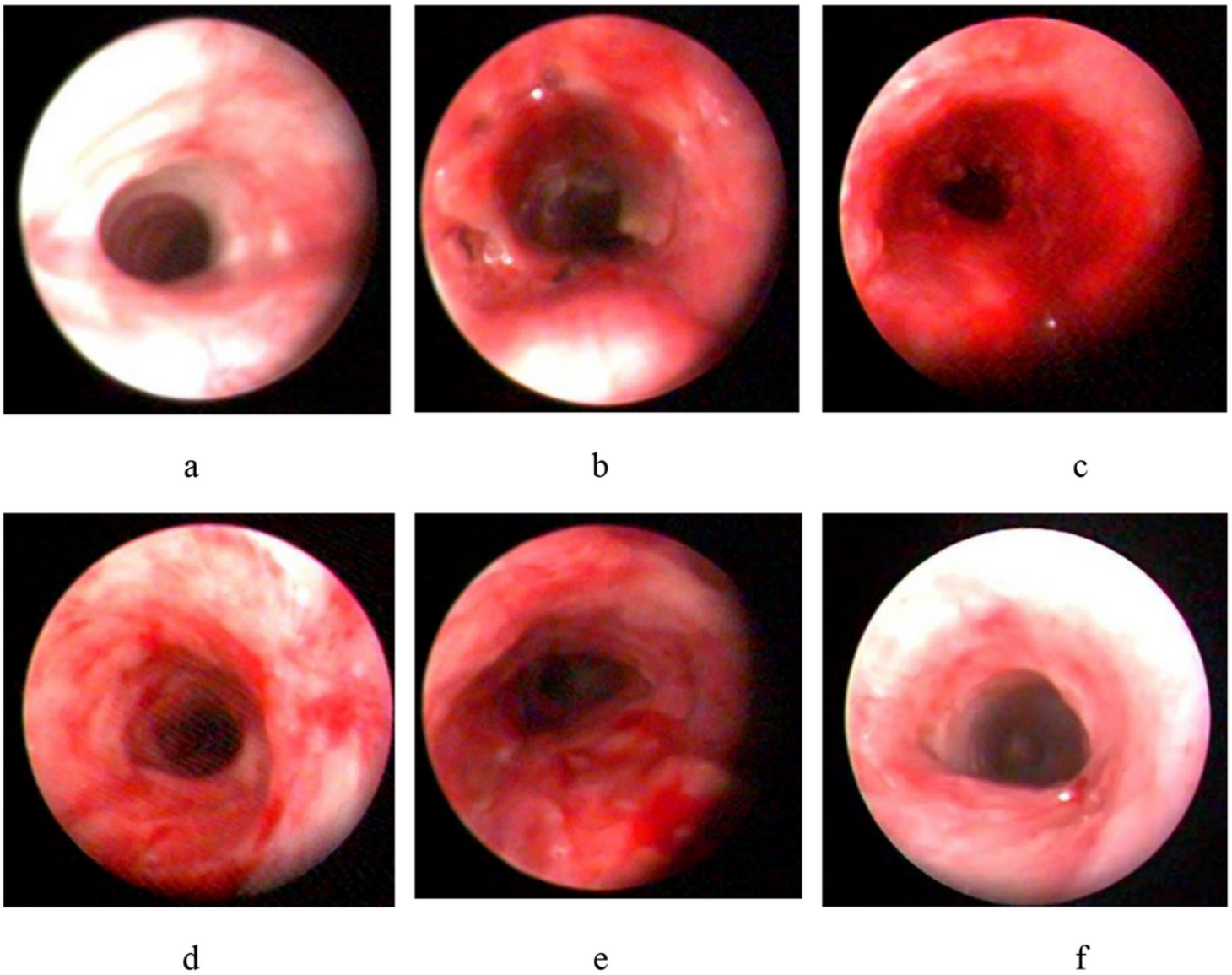

Figure 2

Animal Model of Tracheal Stenosis: RES group (a-c); BMS group (d-f).

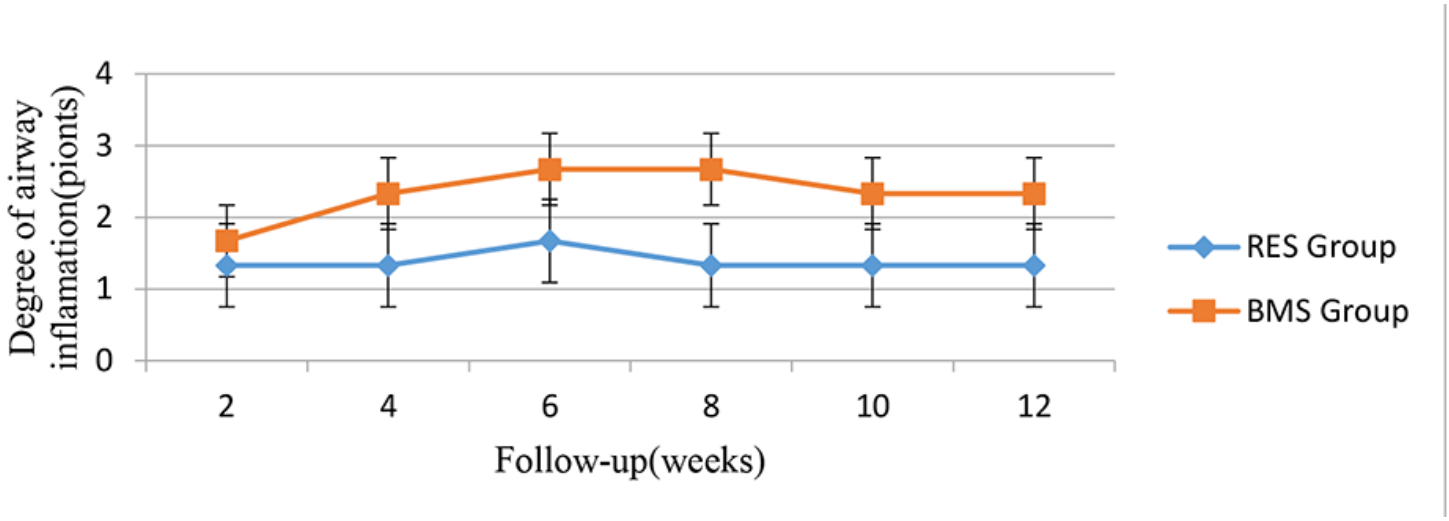

Figure 3

Comparison of airway mucosal inflammation between the RES and BMS groups.

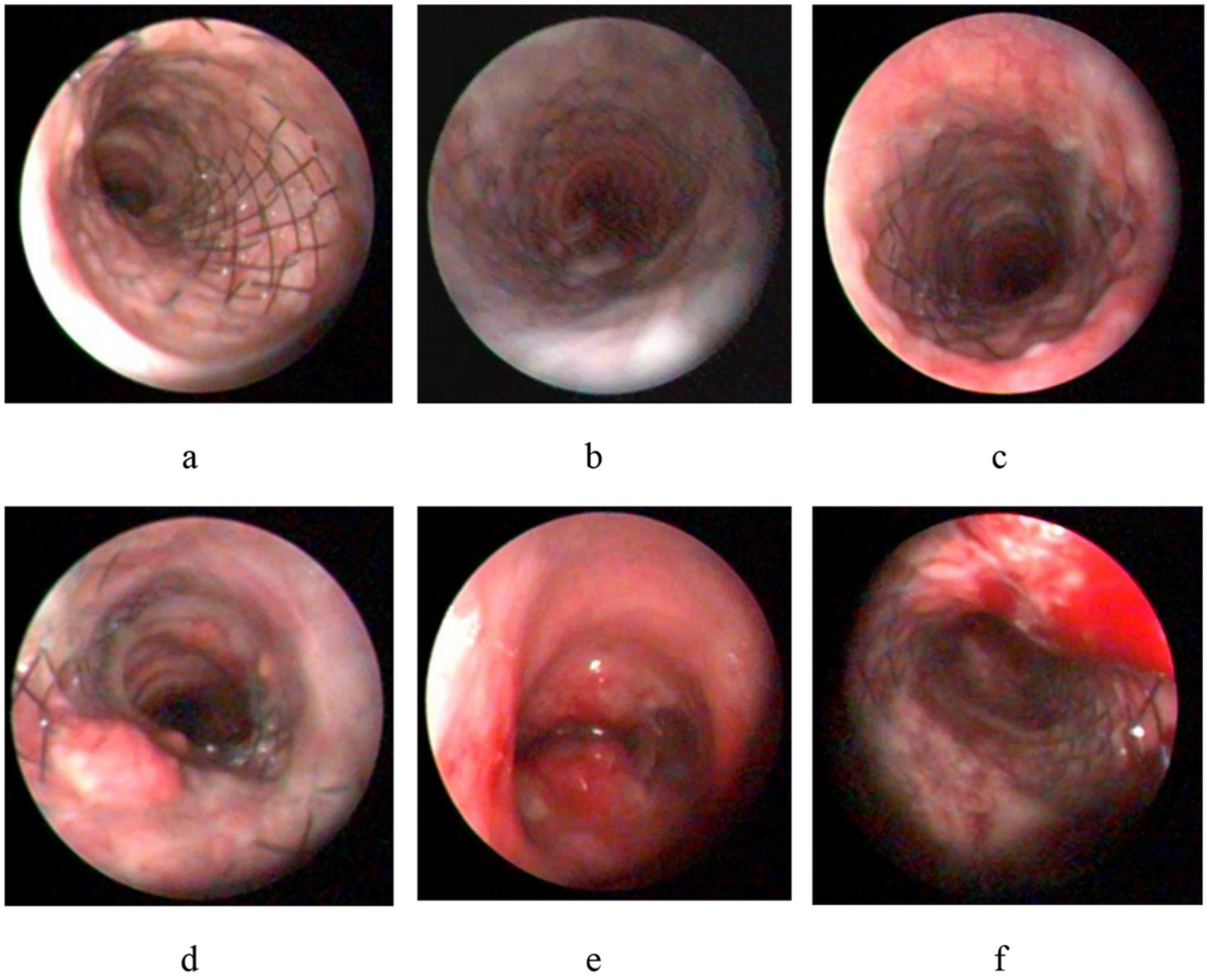

Figure 4

Endoscopic images in the two groups on the 12th week, on the RES group (a-c) and BMS group (d-f).

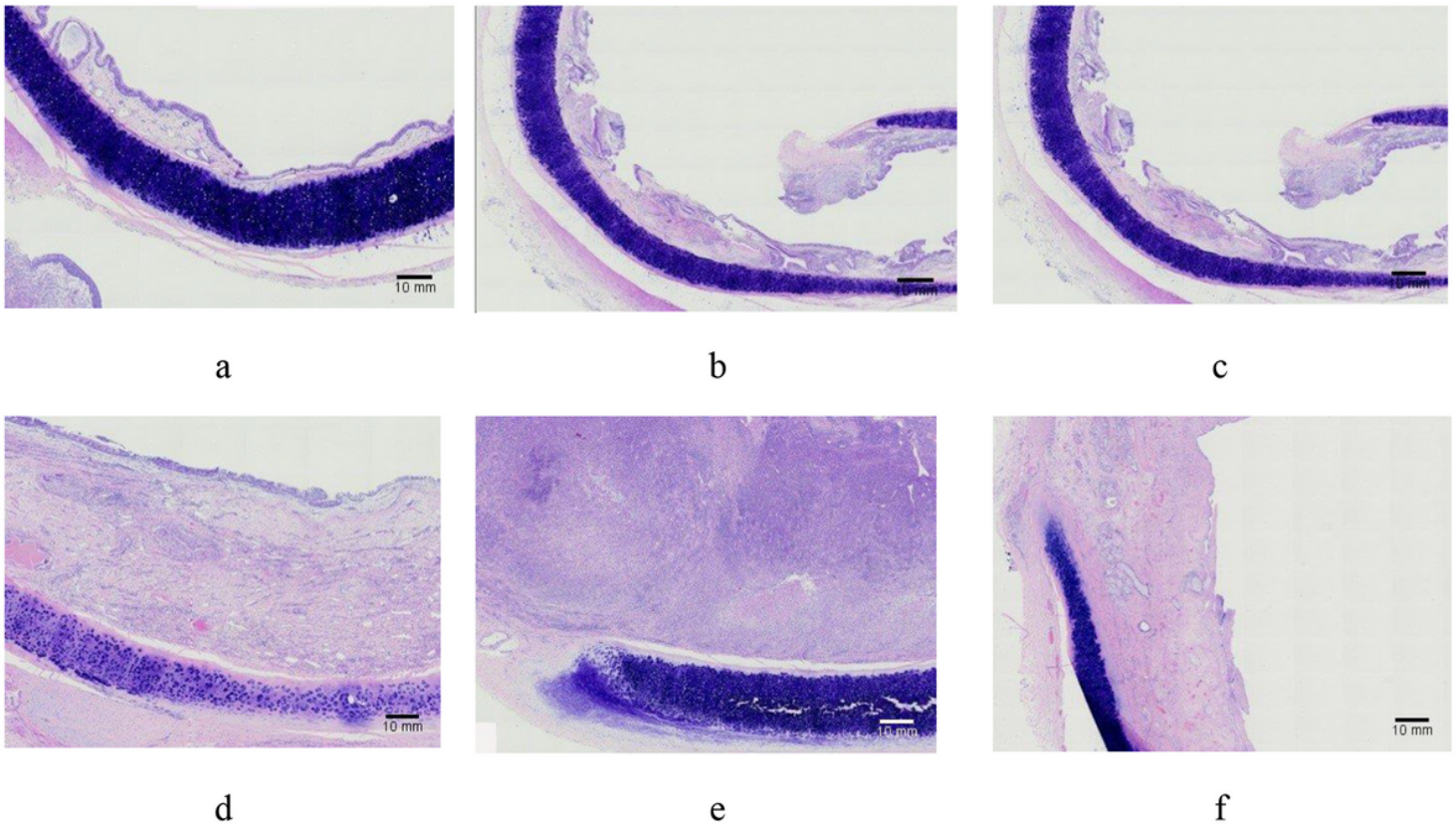

Figure 5

H&E staining (4 cycles) of the stented trachea in the RES group (a-c) and BMS group (d-f).