Lung tissue flap repairs esophagus defection with an inner chitosan tube stent

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Abstract

AIM: To repair the partial esophagus defect with a chitosan stent, a new esophageal prosthesis made of pulmonary tissue with vascular pedicle.

METHODS: Fifteen Japanese big ear white rabbits were divided into experimental group (n = 10) and control group (n = 5). Esophagus defect in rabbits of experimental group was repaired using lung tissue flap with a chitosan tube stent, gross and histological appearance was observed at week 2, 4 and 8 after operation, and barium sulphate X-ray screen was performed at week 10 after operation. Esophagus defect of rabbits in control group was repaired using lung tissue flap with no chitosan tube stent, gross and histological appearance was observed at week 2, 4 and 8 after operation, and barium sulphate X-ray screen was performed at week 10 after operation.

RESULTS: In the experimental group, 6 rabbits survived for over two weeks, the lung tissue flap healed esophageal defection, and squamous metaplasia occurred on the surface of lung tissue flap. At week 10 after operation, barium sulphate examination found that barium was fluent through the esophagus with a slight stricture or back stream, and the creeping was not good at week 10 after operation.

CONCLUSION: Esophagus defect can be repaired using lung tissue flap with an inner chitosan tube stent.

Key words: Lung; Tissue flap; Chitosan; Stent; Esophageal reconstruction

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INTRODUCTION

Esophagus disease is one of the common digestive tract diseases in our country. After excision, the esophagus needs to be reconstructed to restore the digestive tract continuity. At present, the commonly used esophagus reconstruction substitutes mainly include musculo-cutaneous flap with vessel peduncle, platsyma musculo-cutaneous flap, peristome intercostal muscle flap and others from stomach, colon and jejunum. Plastic tube, metallic pipe, teflon tube, silica gel tube, etc, are also used in reconstruction of artificial esophagus. However, their effects are not really ideal. The ideal substitute should be nontoxic, absorbable, without repulsive response and carcinogenicity, and easy to gain.

At present, various esophagus reconstruction techniques are available and each has its own particular advantages and disadvantages. Irrespective of the kind of reconstruction, reducing scar formation and preventing stricture are still the key points. Different flaps from the lung tissue pedial have been successfully applied in reconstruction of trachea.
lung tissue petal, inside lining metal and silica gel pipe stent in animals is also successful, but it is unable to overcome foreign matter response. In this study, we used inside lining metal, a new absorbable biological material-chitosan tube, to repair partial esophagus defect by preventing post-surgery stricture. Some questions concerning its application were discussed.

**MATERIALS AND METHODS**

**Experimental animals and main materials**

Fifteen healthy Japanese big ear white rabbits, weighing 3 kg, were provided by China Medical University Shengjing Hospital Animal Center. The rabbits were divided into experimental group (n = 10) and control group (n = 5). Chitosan tube stent, 15 mm long, 4 mm in inside diameter, was purchased from Shandong Province, China. Antiseptic glutaric dialdehyde was independently developed by the authors. TKR-200C micro-organism life-support machine was provided by Jiangxi Province, China. Rabbit surgery table, chest surgery instruments, infusion instruments, 20% urethane vein anesthetics, ketamine anaesthetics, 1% procaine, laryngoscope, 3.0-4.0 model trachea intubation were bought from Jiangxi Province, China. Tooth pad, medical adhesive plaster, digital camera (Sony, DSC-T10), and optical microscope (Olympics CH-20) were used in this study.

**Experimental techniques**

Before surgery, rabbits did not receive any medicine. Twenty percent of urethane vein anesthetics (5 mL/kg) was injected into abdominal cavity. Three minutes after anesthesia, the breath of rabbits was slow and changed to shallow and abdominal breath with corneal reflex. Ketamine (1 mg/kg) was intramuscularly injected to reduce the pain. The anesthetized rabbits were fixed on the operation table at a supine position, and the assistant pulled in the flank with the bandage to draw in the operation table. The first step was to cut open pleural membrane. When lung collapse was observed, the rabbits were given machinery ventilation, and the breath frequency was adjusted to 30 times/min, and then adjusted according to the lung inflation. A stomach tube was inserted into esophagus to support it, and the center-section was searched for its dissociation, and slung with a spun yarn cloth strip. The excision of central esophagus was an esophagus wall, 3 mm in diameter, to make a animal model of partial esophagus wall damage. In the experimental group, a chitosan tube was placed in breakage of the esophagus and fixed with a needle. The esophagus was wrapped by the nearby lung tissue petal to form an encystation in the breakage place, and the edge of esophagus breakage was sutured continuously with a 3-0 silk suture. Then, the stomach tube was withdrawn to release the stress and return the esophagus. The control group did not need any inside lining chitosan tube. No chest internal hemorrhage and lung air leakage were found. Transition to ventilation was performed several times before the last needle was inserted into the pleural membrane. At end of the inspiration, pleural cavity was closed. The rabbits received fluid diet and anti-inflammation treatment, fresh milk and normal diet a week after operation.

**Observation of target**

**Observation of ordinary circumstances:** Survival, feed, body weight, and complication were observed after operation.

**Observation of body:** If the experimental rabbits died in the observation period, prompt postmortem examination was performed to find the cause of death. The animals were killed at weeks 2, 4 and 8 after surgery, respectively. Scar formation and chitosan tube were observed.

**Observation of histology:** The surviving animals were executed at week 2, 4 and 8, respectively. The damaged patching lung tissue was stained with H&E. The growth of lung tissue petal was observed under optical microscope.

**Barium meal:** Ten weeks after operation, esophagus of survived rabbits was observed by barium meal to see whether the esophagus was unobstructed.

**RESULTS**

**Animal survival**

After operation, 6 animals in experimental group, and 4 animals in control group survived, respectively. Five animals and 1 animal died on the same day after operation, 1 animal died due to anesthesia 1 d after operation, 1 animal died of unhealed fistula on day 4 after operation, 1 animal died of infusion accident on day 7 after operation, and 1 animal died of malnutrition at week 2 after operation.

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Observation of ordinary circumstances

Five days after operation, animals in experimental and control groups could have oral diet. The animals were provided with a small amount of food 5 d after operation and normal diet 10 d after operation. During this period, no obvious feed barrier was observed in animals of the experimental group. Three weeks after operation, animals in the control group had a poor appetite. One week after operation, the body weight of all animals decreased about 1-2 kg. The body weight of animals in the control group was still lighter than before operation.

Observation of body

Two weeks after operation, the esophagus damage was observed, the esophagus breakage edge and lung tissue petal were tightly united, accompanying dropsy. The chitosan tube could be touched under the substitute lung tissue petal and was soft, and the lumen surface had membranous contamination. Four weeks after operation, the damage was completely repaired, the suture was not absorbed. In the experimental group, the internal lumen surface of esophagus substitute was smooth, the blood circulation was rich, and the chitosan tube was partly decomposed. In the control group, the damage was red in color, hyperplasia was found around the damage, accompanying dropsy. In the experimental group, eight weeks after operation, the esophagus defect was covered by the white thick membranous substance. No dropsy, necrosis and ulcer, obvious stricture, or chitosan cast were found in the pipe wall. In the control group, scar formation and hyperplasia were found on tissue petal surface (Figure 1).

Two weeks after operation, the lung structure of rabbits in experimental and control groups was observed. Pulmonary alveoli were withered and collapsed, and the denatured pulmonary alveolus cells were tumescent with acute inflammation response. Four weeks after operation, atypical pulmonary alveolus structure was observed in the experimental group. The inflammatory response was weakened accompanying a few neutral granular cells and lymphocytes, but no obvious fiber proliferation was observed. In the control group, the central pulmonary alveolus structure was atypical accompanying fiber cells and a few inflammatory cells. Eight weeks after operation, a large number of squamous epidermis cells were observed on the surface of lung tissue petal, and the chronic inflammatory response was significantly decreased in the experimental group. The control group had chronic inflammation response, accompanying obvious fiber proliferation but no superficial squamous metaplasia (Figure 2).

Barium meal

Ten weeks after operation, esophagus barium test was performed. In the experimental group, barium meal went smoothly through the esophagus. No obvious stricture, reverse flow, anastomotic stoma leakage and expansion were observed in the esophagus. The creeping motion was good. Mild stricture was observed in control group. The anastomotic stoma was healed with expansion, and the barium meal went through smoothly with general creeping motion (Figure 3).
DISCUSSION

Esophagus excision and reconstruction are important in esophagus surgery. In recent years, biological substitutes have been extensively studied\(^{[13-21]}\). Zhi et al.\(^{[13]}\) reported that biological artificial esophagus can be used to repair esophagus defect. In their study, esophagus substitution test was performed in 30 experimental dogs, showing that esophagus substitution can repair 93.3\% of esophagus defects. Zhang et al.\(^{[14-17]}\) excised chest esophagus at the right chest flank of 30 Chinese hybrid dogs, and an 8 cm long biological artificial esophagus was used to reconstruct esophagus. At present, we still face the problems of fistula, stricture, and length of implants, which need further research. Based on the biological esophagus substitute, biodegradable and non-biodegradable materials have been developed for making artificial esophagus\(^{[18,19]}\). Meanwhile, the degradation speed of biodegradable material matches that of non-degradation material, which exerts the supporting function and prolongs the supporting time of biological materials, and finally regenerates esophagus to completely substitute the artificial esophagus. The project of digestion tube has made certain progress. However, its effect is unknown. Further study is needed on implantation and production of epithelial and muscle cells, gland and nerve plexus regeneration, scar reduction, speeding up repair and regeneration of

Figure 2 Lung tissue flap change under optical microscope in control group (A-C) and experimental group (D-F) after operation (HE, × 10).

Figure 3 Results of esophagus barium test in control group (A) and experimental group (B) ten weeks after operation.
and no immunogenic ability. The... can prevent the adhesion to peritoneum and thus can
breakage site. It was reported that chitosan membrane
can prevent the adhesion to peritoneum and thus can
be used in clinical practice[26]. It was also reported that
chitosan can insert into nerve tube stent[27,28]. Chitosan
tube stent is degraded gradually and absorbed in vivo,
with no toxicity, stimulation and rejection. It has been
shown that epidermis of the esophagus has certain
degree of stricture but it is not serious enough to cause
feed barrier in esophagus of dogs[29]. Long-term survival
and delayed chronic inflammation have been achieved
using metallic pipe and silicone gel tube as an inner lining
support, but foreign matter rejection occurs. We used
chitosan as a support to prevent scar formation and
stricture of esophagus by making use of its compatibility
and degradability. When stricture is formed, chitosan
tube is degraded in vivo, and its product is not toxic
and has no side effects. Thus, it is worthy to be further
studied. The most serious complication is fistula, which
occurs 5-7 d after operation and is related with infection,
anastomotic techniques and blood circulation, etc.
Infection is the most important factor for the occurrence
of complication, because esophagus patching is a
pollutant. Since we performed the surgery under strict
asepsis, we solved the problem. After operation, the
rabbits in experimental group were fasted for 5 d, and
then received venous transfusion and anti-inflammation
treatment. Because the resistance of rabbits was lower
than that of dogs, the mortality rate of rabbits was
30%. Since this study was to verify the new method and
explore experimental conditions, a large sample size
of slightly bigger animals, like dogs, pigs, etc, should be
used in the study. We used big white rabbits to establish
esophagus partial damage model, and repaired esophagus
wall partial damage using chitosan tube with lung
tissue as its inside lining. Based on results of this
experiment, pigs and dogs and other bigger animals, may
be further tested for the replacement of chitosan tube in
the entire esophagus. Our experiment did not operate
chest of rabbits. Compared with big animals, rabbits
are docile, convenient, inexpensive, and easy to obtain.
The choice of support is the key to the maintenance
of unobstructed lumen, protection of the surface
granulation tissue of lung from adhesion, formation of
diverticulum and false passage. The metal lattice support
is widely applied in treatment of esophagus stenosis,
mainly because of its good support effect. Therefore,
we used the chitosan tubular support, which functions
as a support, reduces inflammation response, and can be
absorbed and degraded by organisms, and can be sued as
a substitute of esophagus.

Further study involving smooth muscle, nerve plexus
and gland regeneration is needed[30]. Creeping motion
restoration, long esophagus reconstruction, etc, can be
achieved in clinical practice.

COMMENTS

Background

At present, esophagus reconstruction techniques are available, but each of
them has its advantages and disadvantages. Reducing scar formation and
stricture is still the key to esophagus reconstruction.

Research frontiers

In this study, chitosan tube was used as a support to prevent post-operation
stricture.

Innovations and breakthroughs

Trachea was reconstructed using chitosan and silica gel pipe stent. A chitosan
material was used to make absorbable support tubes to overcome the rejection
and prevent stricture of esophagus.

Applications

Chitosan tubes provide a new surgery method for patients in whom substituting
esophagus with cavity internal organs is impossible.

Terminology

Lung tissue flap: A lung lobe closing the segment of trachea.

Peer review

It is an interesting animal experiment about the use of chitosan stent with a lung
tissue flap to repair esophageal perforation.

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