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Experimental reconstruction of dog's esophagus with biotype artificial esophagus

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OBJECTIVE: At present, there are few materials available for esophagus reconstruction anywhere in the world. The reported survival rate in animals during the perioperative period is comparatively low. The present study assessed the feasibility of using a biotype artificial esophagus in the reconstruction of a dog's esophagus.

METHODS: In 30 mongrel dogs, a portion of the thoracic esophagus was resected and an 8 cm section of artificial esophagus was transplanted to reconstruct the organ. The survival rate, food intake and process of healing were observed.

RESULTS: Of the 30 dogs, 28 survived the perioperative period (93.3% survival). Two dogs (6.7%) developed an anastomotic fistula; 19 dogs survived for 1 year, a survival rate of 79.2% (19/24) with the remaining six dogs were killed according to the experimental protocol. Detachment of the artificial esophagus occurred on average 28.8 days after operation and the dogs suffered from varying degrees of dysphagia 23–45 days after operation. Gradual remission occurred after 4 months. The histological study revealed that the regenerated esophagus was composed of fibrous and connective tissues and the luminal surface was covered with squamous epithelium in 3–6 months.

CONCLUSION: The transplanted artificial esophagus detached after the surrounding 'regenerated esophagus' had formed, and the squamous epithelium gradually covered the luminal surface. Continuous remodeling of the 'regenerated esophagus' gradually relieved the stenosis. Whether detachment of the implant and the postoperative stenosis can be solved is the key problem restricting the use of the biotype artificial esophagus in clinical practice.

KEY WORDS: animal experiment, artificial esophagus, biological materials, esophagus reconstruction.

INTRODUCTION

China has a high incidence of esophageal cancer and operation in the early stage is the best choice of treatment. The main operative technique is to resect the esophageal cancer and reconstruct esophagus with parts of the stomach, small intestine, or colon. Although the cancer is removed, the wound is large and reflux esophagitis, anastomotic stenosis, effects on heart and lung function and some other complications often happen. Therefore the development of new materials that can be used to reconstruct the esophagus is of great significance for the surgical treatment of esophageal disease. From December 2000 to December 2003 we carried out an experimental reconstruction of the dog's esophagus with a biotype artificial esophagus (developed by Guangdong Biological Technology Co. Ltd).

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MATERIALS AND METHODS

Materials

Biotype artificial esophagus (type I, herewith referred to as the artificial esophagus) developed and provided by Guangdong's Guanhao Biological Technology Co.

Ltd was made from fresh pig aortas via a physical–chemical process (US patent 6106555: Method for tissue fixation). It is non-active biologically, 20 mm in diameter and has 2.5 mm wall thickness (Figure 1). The material accords with the National Drugs Supervision Bureau's standard for transplantation materials for the human body.

Experimental animals

The surgery was carried out in 30 mongrel dogs, 25 males and 5 females, weighing 17.5–25.5 kg (average, 20.5 kg). Conventional examination and quarantine were undertaken before the operation; levamisole was used to eliminate parasites and pentavaccine was injected. We did not set up a control group because of the worldwide shortage of material available for esophagus reconstruction.

Experimental equipment

The equipment comprised an operating theater and relevant equipment (respirator, electric knives etc.), conventional drugs, an Olympus XQ-230 gastroscope and Hitachi 800 mA remote controlled X-ray machine.

Surgical technique

The dogs were fasted for 6 h before operation. After routine skin preparation, 3% sodium pentobarbital was given intravenously (1 mL/kg) and atracurium (1 mg/kg) was used to maintain muscle relaxation. Tracheal intubation and a respirator were used to assist breathing. The incision was made on the right posteriolateral chest, then the thorax was entered through the fourth intercostal space. Approximately 10 cm of thoracic esophagus was removed and an 8 cm section of artificial esophagus was transplanted by end to end anastomosis and the reconstructed esophagus was covered with mediastinal thoracic membrane. Thoracic closed drainage was placed and finally the thorax was closed layer by layer. The dogs were kept fasted for 5 days post operation while intravenous nutrition support (100 mL/kg) and antibiotics were given. On the 6th day after operation, liquid food was given and on the 8th–9th day, semiliquid food was provided. The usual diet of solid food was given 2 weeks post operation.

Gastroscopic examination

Gastroscopic examination was performed at the 4th, 6th and 8th week after operation. Examination and therapeutic gastroscopy were performed when dysphagia and repeated vomiting occurred.

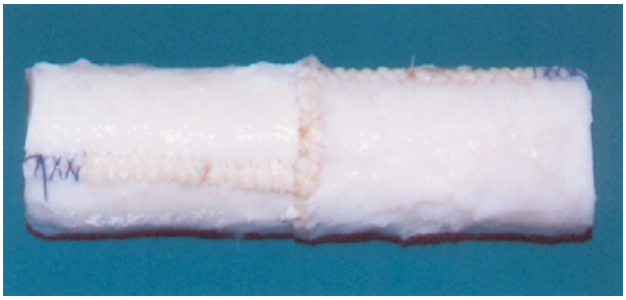

Figure 1. Biotype artificial esophagus.

Histological examination

Two dogs among the survivors were killed in the 1st, 3rd and 6th months. The regenerated section of esophagus was detached via the original incision site and 2 cm of 'regenerated esophagus' from both ends were removed and fixed with formaldehyde. Five samples were taken from every gross specimen: upper anastomotic ostium, 25% of the upper section, the middle point, 25% of the lower section and the lower anastomotic ostium. Samples were cut conventionally and stained with H&E.

Observations

We recorded the following: (i) survival during the perioperative period; (ii) types of complications and the incidence of each; (iii) food intake after operation; (iv) changes in weight; (v) time at which the artificial esophagus dislodged; (vi) growth of the 'regenerated esophagus'; and (vii) the histology of the 'regenerated esophagus'.

RESULTS

Survival rate

Of the 30 dogs, 28 survived the perioperative period (survival rate: 93.3%); 2 dogs died because of anastomotic orifice fistulae, which occurred on the lower anastomotic ostia and on the sides of the esophagus on the 5th and 9th day after operation. There was no leakage from the artificial esophagus. One dog died of lung infection on the 17th day and two dogs suffered from laceration of the regenerated esophagus because of dilation with a probe in the 6th and 8th week and they were later killed. Two surviving dogs were killed after the 1st, 3rd and 6th month post operation, in accordance with the protocol for pathological examination; 19 dogs survived for 1 year (79.2%, 19/24).

Time of detachment of the artificial esophagus

Of the 30 experimental dogs, three died because of anastomotic fistulae and lung infection. In two of the remaining 27 dogs the exfoliated artificial esophagus dislodged into the digestive tract and the exact time of detachment was not known (it occurred 4 weeks after operation during gastroscopy). The time of detachment was ascertained accurately in 25 dogs: two vomited the artificial esophagus, and in the other 23 dogs the implant was removed during gastroscopic examination. In these dogs, the regenerated tissues were found to be connected with the original esophagus, around the transplanted artificial esophagus, which had shrunk and separated from the regenerated one and was able to be drawn out gently with a snare. The time of the detachment of the artificial esophagus was 23–45 days (average 28.8 days) post operation.

Status of food intake

Twenty-seven dogs survived for more than 1 month after operation. All suffered from dysphagia of varying degrees for approximately 1 month (23–45 days). According to the classification of Bown *et al.*[1] (grade 0: intake of all kinds of solid food; grade I: intake of most solid food; grade II: intake of semisolid food only; grade III: intake of liquid food only; grade IV: no intake, even of liquid food), 24 dogs were in grades I and II, accounting for 88.9%. All dogs took food normally after detachment of the artificial esophagus. Among the long-term survivors, some developed dysphagia again 2 months after operation (60–75 days), with 13 (59.1%) in grades I and II, and 9 (40.9%) in grade III. They could take semiliquid food 4–5 months after operation and vomiting was reduced. They resumed normal feeding at 6 months after operation with no further vomiting.

Changes of average weight after operation

The average weight of the 19 long-term survivors was 20.7 kg before operation. Their weights began to fall after operation and reached the lowest point after 2–3 months (18.2 kg). Thereafter, they regained a weight of 20.4 kg at 6 months after operation, which was the preoperative level, and then the weight continued to increase (Figure 2).

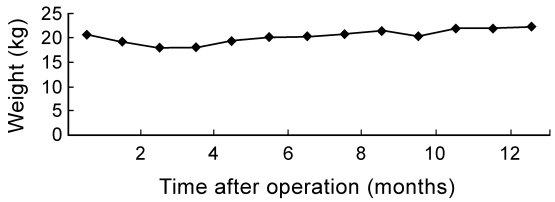

Figure 2. Change in the weights of the experimental dogs before and after operation.

Postoperative stenosis and its treatment

Of the 27 dogs that survived more than 1 month, 18 needed dilation treatment under gastroscopy and 13 of these were only relieved after 2–3 episodes of dilation. Two dogs had to be killed because of esophageal laceration caused by the dilation treatment. Five still suffered from dysphagia after dilation treatment and had a stent installed.[2] These five dogs were cured. None died of malnutrition.

Longitudinal shrinkage of the regenerated esophagus

The 'regenerated esophagus' was found to have shrunk to some extent 3–6 months after operation and was shortened on average by 4.8–5.2 cm (shrinkage of 30–40%).

Gross and histological examinations

One month after operation

Fibrous and connective tissues generally covered the artificial esophagus. The cut surface of the upper and lower sections showed that the fibrous and connective tissues were connected with the original esophagus. The artificial esophagus closely adhered to the fibrous tissues and the ends were separated from the original esophagus by tissue layers of 2–3 mm thickness. The five samples of esophagus were composed of continuous and mixed fibrous and connective tissues with no squamous epithelium on the surface of the lumen.

Three months after operation

Generally, the esophagus was dark red and hard. There was less adhesion around the outer surface than at 1 month after operation. The cut surface showed continuous tissues. The regenerated esophagus section was approximately 6 cm long, with a slightly narrower middle section and dilated upper section. The regenerated esophagus was covered with a thin layer of squamous epithelium. Under microscopy the esophageal samples were composed of fibrous and connective tissues with a thin layer of squamous epithelium on the surface of the lumen.

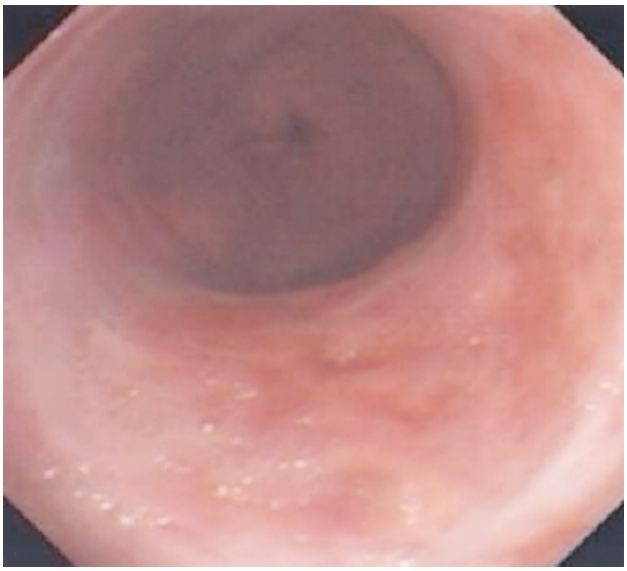

Figure 3. Endoscopy of a dog at 6 months after operation.

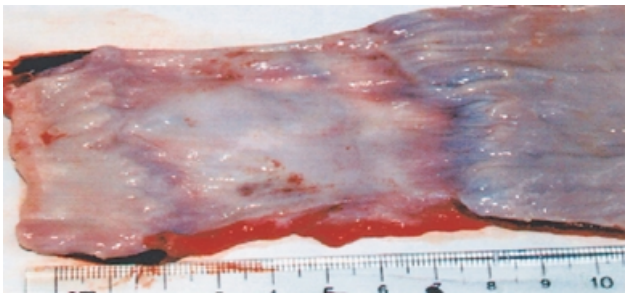

Figure 4. A 'regenerated esophagus' at 6 months after operation.

Six months after operation

Endoscopically the mucosa of the regenerated esophagus was smooth and its color was the same as the original esophagus. The lumen was patent and felt firm when touched with the biopsy forceps (Figure 3). It was an even and unified 'muscular' tract, white in color with a soft texture. The cut surface showed complete epithelium that was connected with the epithelium on the upper and lower sections of the esophagus. The regenerated esophagus segment was approximately 5.8 cm long with a thin wall (Figure 4). Under microscopy the esophageal samples taken from the five points revealed that the surface of the lumen was covered with complete layers of stratified squamous epithelium, beneath which were well-arranged fibrous and connective tissues containing a rich blood supply. Figure 5 shows the histology of the middle section.

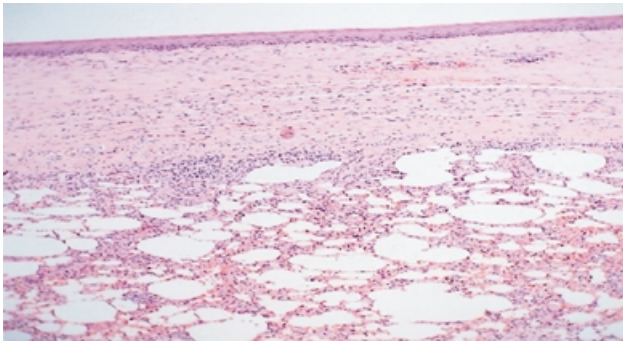

Figure 5. Histology of the middle section of a 'regenerated esophagus' at 6 months after operation.

DISCUSSION

Reconstruction of the esophagus with artificial material has been a research objective throughout the world for several decades. In 1953, Berman used a plastic tube to replace a defective esophagus in the dog and then applied the technique in the human body, but it failed because of complications.[3]

Since 1983, Chinese medical workers have been researching various artificial materials for an artificial esophagus, but have also failed because of poor biological compatibility, inflexibility and unsafe detachment of the material leading to anastomotic fistula as well as postoperative stenosis.[4–8] Miki *et al.*[9] used a polythene reticular brace as the support with attached collagen protein and human squamous epithelium cultured *in vitro*. In an animal experiment they sutured the cultured tissues to a tissue engineered esophagus with a packing of blood vessel muscular valves and inserted the device in the animal. The result was good, but they could not commercialize the technique. We used the biotype artificial esophagus, which has the advantages of good tissue compatibility, soft texture with elasticity, and no danger if the implant detaches from the reconstructed esophagus. The survival rate within the perioperative period was 93.3% in the present study, so this artificial esophagus has good prospects for research, development and future application.

Gastroscopy and the anatomical examination showed that exudation occurred around the artificial esophagus after its transplantation, followed by adhesion to the nearby organs; by covering the artificial esophagus, a fibrous and connective tissue tube (i.e. the 'regenerated esophagus') was thus formed. With proliferation and organization of the fibrous

connective tissue, the density and strength of the regenerated esophagus also increased.

The dogs began to take food 7 days after operation and the continuous stimulation from food intake as well as bacterial action broke the sutures at both ends of the anastomosis. Gastroscopic examination at 2 weeks after operation revealed that the sutures had detached from the original esophagus and the artificial esophagus had shrunk because of the effects of saliva, food and bacteria. Dysphagia occurred to a certain degree. Separation proceeded from the ends toward the center and finally the artificial esophagus was completely separated from the 'regenerated esophagus' and was dislodged into the stomach, digested, vomited out of the body or manually removed during gastroscopy. After detachment of the artificial esophagus, the esophageal lumen became patent and eating became easy. Therefore the transplanted artificial esophagus was only a short-term replacement and support.

After detachment of the artificial esophagus, there was an acute inflammatory reaction (edema, proliferation) in the internal wall of the 'regenerated esophagus'. The squamous epithelial layer at both ends gradually moved towards the center and during this period, some dogs developed dysphagia to some degree because of the inflammation. After approximately 3 months, squamous epithelium covered the whole wall of the esophagus and there was stratified squamous epithelium after 6 months. During this period, the 'regenerated esophagus' was stable and cicatricial proliferation ceased. The circular and longitudinal contraction lessened and the 'regenerated esophagus' gradually became soft and formalized. The 'regenerated esophagus' showed an average of 30–40% longitudinal shrinkage, which was similar to the report by Tian *et al*.[6] Two experimental dogs underwent X-ray examination, which showed smooth passage of the barium, but no peristalsis. The 'regenerated esophagus' was composed of pyknotic fibrous and connective tissue with squamous epithelium covering the surface and many blood vessels could be seen in the fibrous and connective tissues, which indicated the squamous epithelium was stable. Regeneration of muscles and glands has been noted in previous reports,[10,11] but we did not find any evidence in the dogs that survived for more than 6 months. The fibrous and connective tissues were well ordered in those dogs, so the remodeling phenomenon might be related to the stress of food intake.

The main cause of death within the perioperative period of esophagus reconstruction was anastomotic fistula, the incidence of which has been reported as 10–60% in previous reports.[3,8,10] It was 6.7% in the present study, which was much lower than in the other reports and we think the low incidence is related to the material used for the reconstruction and the method of anastomosis. The artificial biological esophagus has good tissue compatibility and is pyknotic, elastic, and soft. Needle punctures close well after suturing so leakage is less likely. Number 0 or 1 nontraumatic suture and 5×17 radian intact round needles were used to perform the sectional, whole layer and end to end anastomosis with two-thirds introversion of the back wall and one-third extroversion of the front wall. Other factors, such as the blood supply of the esophagus, the dog's hygiene and manner of eating, and poor nutritional support after operation might lead to anastomotic fistula. In addition, the dog naturally eats its food rapidly and frequently regurgitates, which makes the lower anastomotic ostium vulnerable to the formation of fistula. This conditions are less likely in humans.

Two dogs dislodged the artificial esophagus into the digestive tract after the perioperative period and another 2 vomited them out. In the remaining 23 dogs (81.5%), the artificial esophagi were removed by a snare during gastroscopy. In most cases detachment of the transplanted esophagi was not smooth, which will be a major issue to overcome in the future.

Another important and difficult problem is the occurrence of stenosis after reconstruction, which directly affects survival. Our experiment showed that stenosis occurred after operation under two conditions: (i) inflammation and granulation tissue hypertrophy of the 'regenerated esophagus' before and after detachment of the artificial esophagus;[12] and (ii) during the period of remodeling of the 'regenerated esophagus'. There are four ways to solve these problems: (i) increase the time the artificial esophagus remains within the body to reduce the reactive inflammation before and after detachment; (ii) feed soft or semiliquid food to reduce the irritation caused by food intake and prevent the overproliferation of granulation tissue; (iii) dilatation treatment, if required for severe stenosis (e.g. 2 months after operation) should be performed gently, starting with the smallest caliber probe and gradually increasing in size; and (iv) an esophageal stent should be inserted if none of the other measures are successful.

The biological artificial esophagus has the following features: (i) non-toxic and good tissue compatibility in accord with the national standard for long-standing transplantion materials into the human body; (ii) the regenerated esophagus will form after the biological artificial esophagus replacement stimulates the production of an epithelium-covered fibrous and connective tissue structure; (iii) stenosis of the 'regenerated esophagus' can be relieved or cured with better diet and medical treatment; and (iv) a simple and safe reconstruction that causes few complications, produces little effect on the heart and lungs and does not interfere with physiology. The dog model showed the healing course as well as the occurrence and outcome of complications and provides data for clinical studies. However, before the biological artificial esophagus (type I) can be used in a clinical trial, the problems of detachment and stenosis after operation must be solved.

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