Growth and Development of Homograft Tracheal Transplants in the Piglet Model

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Objective: To determine the growth characteristics of homograft tracheal transplants in piglets.

Design: Prospective controlled animal study.

Setting: Clinical animal laboratory.

Subjects: Seventeen Yorkshire swine piglets.

Interventions: The tracheae of adult Yorkshire swine were harvested and treated with formaldehyde, thimerosal, and acetone to remove immunogenic major histocompatibility complexes. Eleven piglets had these chemically treated homografts transplanted into 6-cm surgically created tracheal defects. The transplants were stented. Three control piglets had a 6-cm anterior tracheofissure, no transplant, and surgical placement of the stent. Three other control piglets had no transplant, and the stent was placed endoscopically.

Main Outcome Measures: Growth outcome measurements were tracheal length and diameter. Functional outcome measurements were lumen patency and graft viability indicated by cartilage retention.

Congenital tracheal stenosis, characterized by variable lengths of narrowed trachea, can be a life-threatening condition in infants.1 The affected trachea fails to fully canalize during fetal development, resulting in complete tracheal rings with a lumen size of less than 4 mm. Long-segment stenosis exists if the stenotic area is greater than 50% of the tracheal length. Children with congenital long-segment tracheal stenosis usually develop acute respiratory distress within the first year of life, commonly precipitated by an acute upper respiratory tract infection. Oftentimes the first indication of long-segment tracheal stenosis is failure to extubate a pediatric patient because of edema caused by intubation. This anomaly is often associated with cardiovascular defects. Of neonates with congenital stenosis, 25% to 50% have pulmonary vascular slings and 20% to 36% have intracardiac lesions or a right-sided aortic arch.2-5 The first indication of tracheal stenosis might be difficulty intubating a neonate for repair of a cardiovascular defect, thus complicating an already difficult surgical problem. Despite advances in pediatric cardiothoracic surgery and airway reconstruction, congenital tracheal stenosis remains a perplexing surgical challenge.

In newborns, long-segment tracheal stenosis is usually fatal if uncorrected. Surgical treatment is difficult, with mortality rates up to 41%.6 End-to-end anastomosis and tracheoplasty with costal cartilage or pericardial grafting are useful surgical techniques for small tracheal...
MATERIALS AND METHODS

The tracheae of 30 adult Yorkshire swine were harvested. The diameter of the trachea was measured at the middle, and the length was measured from the cricoid to the carina. Future grafts were stripped of mucosa and connective tissue under clean conditions and immersed in 4% formaldehyde solution. After 2 weeks, the grafts were rinsed in sterile saline solution and immersed for 56 days in thimerosal (sodium ethylmercurithiosalicylate), 4 g/L, dissolved in phosphate-buffered saline solution. The grafts were then stored for at least 10 days at aceton. All processing and storage was in autoclavable and acetone-resistant polypropylene bottles (Nalgene, a subsidiary of Sybron, Rochester, NY).

Seventeen piglets with an average weight of 25.6 kg were acclimatized to the animal research facility for 2 weeks before surgery. All animals were treated in compliance with the guidelines, standards, and policies of the Institutional Animal Use and Care Committee. Immediately before surgery, each piglet was premedicated intramuscularly with xylazine hydrochloride, 2.5 mg/kg, and ketamine hydrochloride, 20 mg/kg. One percent lidocaine was topically applied to the larynx, followed by intubation with a cuffed endotracheal tube (inner diameter, 4.5 mm). Anesthesia was maintained with isoflurane. The hyperextended anterior neck was scrubbed, prepared, and draped in a sterile fashion. A midline anterior cervical incision was made from the skin, subcutaneous tissues, and platysma. The strap muscles were retracted and the cervical trachea was exposed. The recurrent nerves were identified and protected. A Penrose drain was placed under the proximal trachea, and the trachea was released from the surrounding tissues. The mediastinal trachea was gently mobilized into the cervical incision by traction on the Penrose drain, and a 6-cm midline incision was made beginning 1 cm below the cricoid. The endotracheal tube was withdrawn, the inner diameter of the trachea was measured, and an 8-cm flexible Silastic stent (Hood Labs, Pembroke, Mass) was inserted into the trachea, taking care to ensure that there was a 1-cm overlap of the stent into the normal trachea on either side of the tracheal defect (Figure 3). The endotracheal tube was advanced into the lumen of the stent, and assisted ventilation continued. The anterior two thirds of the trachea was excised, leaving the trachealis muscle and a minimal amount of cartilage posteroirly. The graft was sutured securely into place with 4-0 polypropylene (Prolene) sutures, and the incision was closed in 3 layers, with a temporary Penrose drain in place until the piglet was extubated (Figure 4). The wound was dressed with a compression dressing, and the piglet recovered. No immunosuppressive medications were given because of previous graft immunosterilization with formaldehyde and thimerosal. Postoperative pain was managed with intramuscular injections of buprenorphine hydrochloride, 0.15 mg/kg.

Piglets were observed with serial telescopic bronchoscopy. The schedule for bronchoscopy included a 2-week postoperative examination and an examination at 2 months for stent removal plus additional bronchoscopies if the animal exhibited stridor. Endoscopic photography was obtained to document healing (Figure 5). Perioperative antibiotics (amoxicillin with clavulanate potassium, 250 mg by mouth 3 times per day) were used until the stent was removed. The pigs were closely monitored for signs and symptoms of airway distress. Animals with airway distress that could not be resolved were humanely killed.

Two months after surgery, the animals were humanely killed under anesthesia using intravenous euthanasia solution containing pentobarbital sodium, 390 mg/mL, and phenytoin sodium, 50 mg/mL, at a dose of 1 mL/4.5 kg. The airway was endoscopically photographed before and after stent removal (Figure 6). The tracheae were harvested, and tracheal lengths and luminal diameters were measured. Representative sections were sent for hematoxylin-eosin staining.

defects, however, when a small child's segmental tracheal stenosis is longer than 5 cm, present surgical techniques commonly fail.

Homograft tracheal transplantation, a promising new surgery being developed in Germany, might provide realistic hope for infants with devastating tracheal stenosis. A cadaveric adult trachea or bronchus is treated with formaldehyde, thimerosal, and acetone and then used to surgically replace or augment a defective trachea.

Tracheal transplantation can provide an enlarged adequate airway, but graft rejection is a significant concern. Major histocompatibility complexes (MHC) are inherent in every cell. These tissue transplant complexes are recognized by the host immune system as foreign. The immune system is then activated, and rejection of the homograft occurs. Immunohistological studies have demonstrated that in humans, the MHC can be removed by immersing tracheal homografts in formaldehyde and thimerosal before transplantation. Irradiated tracheal cartilage has been transplanted successfully into small tracheal defects in rabbits and dogs, and there was no evidence of rejection. Synthetic grafts have been investigated, but these are uniformly unsuccessful because of the problem of infection. Other animal studies have investigated microvascular grafting, cryopreservation, and homografting after denervation of the spleen. Summarized, the results of these studies show that it is possible to transplant homograft tracheae into pigs, dogs, rabbits, and humans; however, unless the MHC markers are removed, or the recipient is immunosuppressed, rejection will occur. Cryopreservation, irradiation, and chemical preservation effectively remove the MHC markers and allow airway transplantation without immunosuppression.

We are presently following up a 6-year-old child who received the first human tracheal transplant for long-segment tracheal stenosis as an infant (Figure 1). The graft has been in place for almost 3 years, and the child has minimal respiratory symptoms; however, endoscopy reveals little evidence of tracheal growth, and the transplanted segment is malacic (Figure 2). Other institutions have begun to transplant homograft tracheae into infants using this technique as well. Can we expect the transplanted trachea to grow with these children, or will further procedures be required to enlarge the trachea? This study answers the question of growth in a pig-
let model using the same techniques that are currently achieving success repairing long-segment tracheal stenosis in adult humans.

RESULTS

Eleven piglets received homografts and survived longer than 40 days (Table). The grafted segments of the homograft tracheal transplants had an average diameter of 6.64 mm at the time of harvest. The tracheofissure control group had an average diameter of 11.00 mm, and the endoscopic control group had an average diameter of 14.00 mm (Figure 7). The average length of the homograft transplant tracheae was 19.27 cm compared with 19.00 cm for the tracheofissure control group and 15.00 cm for the endoscopic control group.

All of the tracheae that received homograft tracheal transplants were malacic (Figure 8). Five animals in the homograft transplant group died after experiencing stent migration followed by airway compromise due to small airway diameter and tracheomalacia. The remaining 6 animals in the transplant group were humanely killed within 4 hours of stent removal because they experienced airway compromise due to small airway diameter and tracheomalacia.

Histologic examination showed progressive resorption of the graft cartilage without evidence of rejection (Figure 9) and eventual replacement with collagen. The epithelial architecture of the grafted specimens progressed from squamous metaplasia, through cuboidal, to pseudostratified columnar epithelium without cilia. The 3 animals with surgically placed stents had endoscopically visible scarring with histologically normal mucosa. The trachea at the site of tracheofissure was rigid. The 3 animals with endoscopically placed stents had no significant scarring and histologically normal mucosa. Four of the 11 grafted tracheae showed small isolated pockets of neochondrification in the submucosa.

COMMENT

The observation of malacia in our human infant homograft case stimulated interest in the growth potential of this technique. The Yorkshire swine grows rapidly and
was chosen so that tracheal growth could be studied over a short time. Yorkshire swine piglets grow to an adult size at approximately 6 months of age. Normal tracheae of Yorkshire swine piglets grow from an average length of 11.0 cm and a diameter of 11.5 mm at 6 weeks of age to an average length of 18.6 cm and a

Figure 5. A, Rigid bronchoscopic placement of a stent into the trachea of a control piglet. B, Endoscopic control after removal of the stent at 2 months. Note the tracheal granulations from the posts after removal of the stent. C, A piglet from the tracheofissure control group after removal of the stent 2 months after surgery. Note the teardrop shape of the lumen resulting from the anterior tracheofissure. D, Two weeks after surgical placement of the graft and stent. The airway remains patent, with no appreciable granulation.

Figure 6. A, Homograft with stent at 2 months. The native trachea has enlarged, and the grafted segment shows no growth. B, Homograft immediately after stent removal. The airway is severely malacic, with granulation tissue, and is incompatible with life.
diameter of 20.0 mm at 6 months of age. In this study, the tracheae transplanted with homografts reached normal length, but the diameter of the lumen was almost half of the preoperative diameter and clearly not approaching the normal tracheal diameter. In addition to the smaller lumen, the transplanted segments were malacic, which can be attributed to the resorption of the graft cartilage and subsequent replacement with collagen. The small amounts of neo-chondroplasia that we saw were not enough to provide any rigidity 2 months after surgery, but it is conceivable that if that process continued, the malacia might be reduced. It is encouraging that epithelialization occurred in many of the tracheae. The lack of cilia in these samples should be expected because the movement of the stents within the trachea will traumatically remove the cilia.

The rapid growth of the normal tracheae in these piglets posed a problem for stent retention that might not be mirrored in growing children. As the normal tracheae enlarged around the grafted segments, the stents tended to migrate. In a child who does not grow as rapidly, the stent might be retained longer with a lower risk of stent migration. Longer retention of the stent might allow maturation of the scar, which would provide a more rigid trachea, but it is unlikely that the tracheal lumen will enlarge. Additional operations might be required in children with homograft tracheal transplants to provide them with an adequate airway as they grow into adulthood.

Homograft tracheal transplantation has been performed in children and adults. The long-term results of transplantation in children are unknown. This study suggests that homograft tracheal transplantation will result in a collagen scar replacing the graft and that the grafted segment will tend to contract with further maturation of the scar.

![Figure 7. Airways of the endoscopic control group had an average transverse diameter of 14.00 mm (left). Airways of the tracheofissure control group had an average transverse diameter of 11.00 mm (right).](https://jamanetwork.com/)

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*Preop indicates preoperative; postop, postoperative; native postop, unoperated-on trachea distal to graft or tracheofissure site measured on day animals were killed; DOS, day of surgery; and DOD, day of death.*
tion. There was no evidence of host-vs-graft rejection, but the graft cartilage is resorbed, resulting in tracheomalacia. As a result of the small lumen and tracheomalacia, the trachea was incapable of supporting respiration. These findings suggest that homograft tracheal transplantation might result in a small malacic airway with little potential for growth when performed in children.

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REFERENCES