Use of the Peritracheal Fold in the Dog Tracheal Transplantation Model

Patrick J. Gannon, PhD; Peter D. Costantino, MD; Edgar A. Lueg, MD; John M. Chaplin, MB, ChB; Margaret S. Brandwein, MD; Philip J. Passalaqua, MD; Lawrence J. Fliegelman, MD; Jeffrey T. Laitman, PhD; Samuel Marquez, MPhil; Mark L. Urken, MD

Objective: To investigate the technical aspects of the canine model of human tracheal transplantation for potential application to reconstruction of extremely long tracheal defects (>10 cm).

Design: In phase 1, long tracheal segments were skeletonized and pedicled with the thyroid glands, cranial thyroid arteries and veins, and internal jugular vein branches. The segments were elevated completely, attached to the vascular pedicle only, and replaced with primary tracheal anastomoses. In phase 2, long segments were elevated along with a diffuse soft tissue “blanket” that envelops the trachea and thyroid glands. Because this study was designed to primarily address, in situ, tracheal perfusion territories of a cranially located vascular pedicle, microvascular anastomoses were not conducted.

Subjects: Two small-bodied beagles (10-15 kg) and 5 large-bodied mixed-breed dogs (20-30 kg) were humanely killed 2 to 41 days after surgery, and anatomic and histological analyses were conducted.

Results: Unlike that of humans, the thyroid gland complex of dogs is not intimately associated with the trachea but is conjoined with a peritracheal soft tissue “fold.” Within this fold, blood is transmitted to the trachea via a diffuse, segmental vascular plexus. In phase 1, pronounced tracheal necrosis occurred within 2 to 5 days. In phase 2, extremely long tracheal segments (10-12 cm), based only on a cranially located pedicle, were still viable at 2 to 6 weeks.

Conclusions: Preservation of the “peritracheal fold” in the dog model of tracheal transplantation is critical to the onset and maintenance of vascular perfusion in a long tracheal segment. Furthermore, the use of large-bodied dogs is necessary to provide for a usable venous efflux component.


At present, no reliable method is available for reconstructing defects that result from resection of tracheal segments larger than 6 cm. Short-segment tracheal defects (~4 cm) may be more readily managed by resection and primary anastomosis, staged reconstruction, or techniques such as slide tracheoplasty. Although results of an early cadaver-based study supported the feasibility of primary tracheal anastomosis in defects as long as approximately 6 cm, they did not account for the myriad of factors present in the clinical setting. Use of stents is associated with many complications and currently represents only a form of palliation. Other approaches—use of cadaveric allograft tracheas or autogenous grafts such as omentum, myoperiostium, pericardium, or cartilage—are not readily applicable to permanent repair of long-segment tracheal defects.

Recent advances in the reliability of free tissue transfer techniques may allow tracheal transplantation to be applied. An early case study reported successful transplantation of a tracheal allograft in 1 patient. Before this method can be applied with confidence to humans, it is necessary to demonstrate its efficacy in an animal model. This study was designed to take a new look at a previously used but problematic canine model of human tracheal transplantation.

A study that used microvascular techniques for tracheal transplantation in small-bodied dogs (beagles) with vascularized tracheal allografts (12 rings, ~4.5 cm) showed some success. Although similar to this study, the cranial thyroid artery (CTa) served as the primary arterial blood supply to the transplanted segment; no provision was made for venous outflow. This was likely because it was not possible to readily conduct microvascular anastomoses of the extremely small
MATERIALS AND METHODS

The size of the transplanted organ—thus, the animal model species of choice—is likely an important factor because it pertains directly to variables related to the spatial dynamics of tissue perfusion. Dogs allow for the use of an essentially human-sized cross-sectional area of the tracheal lumen. The dog trachea comprises 30 to 32 rings, compared with 16 to 20 in humans, thus allowing even longer cervical tracheal segments to be used. Furthermore, results of dye perfusion studies\(^2\)\(^2\)\(^3\)\(^4\) in humans, dogs, and baboons show that analogous vessels, the inferior thyroid, and CTa’s supply a similarly sized tracheal vascular territory. This study was conducted in 7 dogs (*Canis familiaris*; 2 small-bodied beagles, 8-12 kg [an animal model used previously in other studies], and 5 large-bodied mixed breed, 25-30 kg). Animals were treated in accordance with guidelines of the Institutional Animal Care and Usage Committee at Mount Sinai School of Medicine, New York, NY. Animals were anesthetized using sodium pentobarbital (15 mg/kg intravenously) and endotracheally intubated. Using a sterile technique, a vertical midline neck skin incision was made and the infrahyoid muscles were separated and retracted, exposing a large portion of the trachea (~18 rings) and larynx. All branches of the CTa and CTv with distribution to the larynx and infrahyoid muscles were ligated (Ligaclip stainless steel ligating clip LS-100; Ethicon, Somerville, NJ). Descending branches of the CTa and CTv to the thyroid gland and trachea were maintained. The trachea was divided caudally; the distal segment was intubated with a second endotracheal tube and the cranial portion was divided. When fully elevated and connected by only the vascular pedicles (*Figure 1*, B), the trachea was placed back into position. Primary tracheal anastomoses were performed cranially and caudally using circumferential, interrupted polypropylene sutures (2-0 Prolene; Ethicon) that were placed sequentially and tied concurrently to distribute the lumenal tension equally. The infrahyoid muscles were sutured together loosely in the midline, and the wound was closed in layers using interrupted sutures. Animals received antibiotic drugs (cefazolin sodium, 25 mg/kg subcutaneously, twice daily for 7 days), glucocorticoids (dexamethasone sodium phosphate, 4 mg/kg intramuscularly, twice daily for 2 days), and analgesic medications (butorphanol tartrate, 0.4 mg/kg subcutaneously, twice daily for 2 days).

In phase 1 (beagles), in a similar manner as may be used for humans, all peripheral tissues associated with the trachea other than the thyroid gland and associated vessels were dissected away and retracted laterally, and descending branches of the CTa and CTv were exposed bilaterally. This technique was similar to that used previously on beagles.\(^1\)\(^3\) Superiorly, the trachea was divided between rings 1 and 2 and at the appropriate level inferiorly to elevate long tracheal segments containing 9 and 11 rings, respectively, in the 2 dogs. In 1 animal, a small (~0.5 mm), caudally extending branch of the external jugular vein was also maintained.

A new surgical approach for phase 2 was formulated, based on technical experience and results obtained during phase 1. In these large-bodied mixed-breed animals, soft tissues associated with the thyroid gland and trachea, the “peri-tracheal fold” (PTF) (*Figure 1, A*), were meticulously preserved. The carotid arteries and recurrent laryngeal nerves were freed dorsally. Recurrent laryngeal nerves were carefully dissected away within the PTF from tracheal ring 20 to the level of the larynx, with effort made to maintain vascular integrity. A 15-ring segment of trachea (10-12 cm)—pedicled on the CTa, CTv, and branches of the internal jugular veins bilaterally—was elevated (*Figure 1, B*). In all cases, when brisk bleeding occurred from both ends, the tracheal segment was placed back into position and Anastomosed primarily.

At 13 to 41 days, animals were humanely killed (pentobarbital, 0.26 mg/mL, 10 mL intravenously) and tracheal specimens were removed for staged dissection, a photographic record, and histopathologic analysis. Multiple representative tracheal tissue specimens were removed, prepared for histologic analysis, and assessed by an otolaryngological pathologist (M.S.B.) for multiple factors, including avascular necrosis, cartilage regeneration, inflammatory processes, mucosal viability, vascular compromise, and estimates of cartilage cell loss (based on an estimate of percentage of chondrocyte loss). Because tracheal tissues of dogs in phase 1 were severely necrotic, it was unnecessary to conduct histopathologic analysis.

(<0.5 mm, see the “Results” section) associated venous elements such as the cranial thyroid vein (CTv) and internal jugular veins\(^6\)\(^7\) in these small-bodied dogs. In addition, the transplanted tracheal segment used was within the range of tracheal defects in humans that can be addressed by primary anastomoses. Other studies have used canine models to explore vascular augmentation of tracheal autografts. For example, Nakanishi et al\(^17\) reported difficulty in restoring blood perfusion to a graft because the tracheal vessels were too fine. To augment the blood supply to the grafted trachea, they wrapped the tracheal autografts in an introduced portion of pedicled greater omentum. However, even with omental augmentation, grafts longer than 4 cm developed extensive avascular necrosis. Yokomise et al\(^18\) also used omental augmentation of nonvascularized tracheal allografts to evaluate pretransplantation irradiation vs immunosuppression. Recently, they presented an omentopexy-based procedure for use of longer (multiple and serial) tracheal segments by splitting the grafts in their middle portion.\(^19\) Although variants of the omentopexy approach to tracheal transplantation have had moderate success, segments longer than 4 cm still require an additional vascular source.\(^20\)\(^21\)

RESULTS

In phase 1 studies, conducted in small-bodied beagles, CTa diameters were less than 0.8 mm, and CTv’s were either not apparent or less than 0.5 mm in diameter. After tracheal elevation, bleeding from the transected extremities of the tracheal segment was minimal, even after local topical application of a vasodilator agent (papaverine hydrochloride, 30 mg/mL). Both animals exhibited pronounced respiratory stridor 1 to 2 days after surgery. At 2 days, because there was severe breakdown
of both tracheal anastomotic sites and necrosis was apparent along the entire tracheal segment, animals were humanely killed. The segment was dehiscent at multiple sites, with pronounced sloughing of the lumenal mucosa. In both dogs, the CTa and CTv pedicles were intact, although blood flow to and through the thyroid gland was compromised.

Unlike in humans, the dog thyroid gland and its associated vasculature did not have an intimate anatomic association with the trachea but instead were anatomically related to a diffuse, pervasive soft tissue “fold.” The main branches of the CTa that passed through the thyroid gland ramified within this structure. In turn, these branches distributed to the trachea in the form of a diffuse radiate microvascular plexus that supplied blood in a segmental manner to a caudal level of at least tracheal ring 20. We termed this previously unreported structure the PTF. This finding led us to develop an alternate surgical approach, which was applied in phase 2. Unlike animals in phase 1, when the PTF was preserved in phase 2, bleeding from the caudal and cranial transected extremity of the tracheal segment was brisk and was enhanced by local topical application of a vasodilator (papaverine). Although a microvascular anastomosis component was not used in this study to minimize variables, it became clear that the previously used small-bodied beagle would not be suitable for planned future studies. This was largely because of the small size of the vessels in the chosen vascular pedicle. However, in the larger bodied dogs in phase 2, the CTa’s and CTv’s were considerably larger (>1.3 mm), and several tracheal branches to the small internal jugular veins were evident bilaterally. Although the distribution of these venous elements was highly variable, in all cases, at least 1 anastomosable vessel was present bilaterally.

Phase 2 animals were killed humanely at 13, 18, 29, 35, and 41 days. Results of gross anatomic dissection analyses demonstrated that (1) the PTF had maintained its intimate vascular relations with the trachea (Figure 2, A-C); (2) the cranial and caudal sites of primary tracheal anastomosis were well healed (Figure 2, C); (3) although ring overlap and some minimal “webbing” was often present at the site of primary tracheal anastomosis (Figure 2, B), there was no evidence that this could have become symptomatic because minimal inflammation was present; and (4) the tracheal mucosa was intact along the entire length of the graft, including crani-
Preservation of what we termed the PTF in dogs is critical for the survival of long tracheal segments supplied solely by a cranially located vascular arterial pedicle. In fact, our results show that an extremely long segment of trachea (>15 rings, 10-12 cm) can be perfused by bilateral vascular pedicles situated cranially. Results of previous tracheal dye perfusion studies of this vascular pedicle in dog cadavers prepared by transcardiac perfusion fixation—in which all blood is flushed out of the vasculature—showed that extensive bilateral vascular collateralization occurs. Results of a similar study conducted in human cadavers also indicated that this bilateral collateral network was present and robust. These previous findings indicate that, even if an anastomosed arterovenous pedicle failed on 1 side, the entire bilateral long tracheal segment would survive, based on perfusion from the single pedicle. This study did not use microvascular anastomoses because it was considered a distinct variable that was not directly relevant to the question of in vivo (pedicle-based) tracheal vascular perfusion territories being addressed.

A new anatomic finding incorporated into this study involved characterization of the venous elements involved with the trachea. Previous studies were unable to provide adequate venous egress because the vessels associated with the trachea and PTF are small and diffuse, particularly in small-bodied dogs such as beagles. However, we used anastomosable-sized vessels as they emanated from the PTF to join the internal jugular veins and vena comitans with the external jugular system. This methodological advance allows adequate venous egress to be established throughout the entire long segment of trachea.

Results of gross and microscopic examination did not indicate compromise of cartilaginous elements (ie, no increased chondrocyte dropout or frank necrosis) in the central portion of the tracheal segment. The middle graft site is often considered to be at highest risk because it is furthest from viable host tissues, where rapid ingrowth of new blood vessels during the first several days might enhance the viability of proximal graft sites. In phase 1, where the blood supply to the tracheal segment via the vascular pedicle was clearly not sufficient, there was no indication of any significant vascular contribution (via neovascular ingrowth) to the ends of the elevated tracheal segment from the adjacent cranial and caudal tracheal portions. In fact, both phase 1 animals showed frank necrosis throughout this site after 2 postoperative days. However, even in phase 2, in which the vascular pedicles and PTF might have supplied a more-than-sufficient supply of blood to the segment, there was some as yet anecdotal visual evidence of increased mucosal vascularity of the tracheal segment adjacent to the anastomotic sites (Figure 2, C).

The use of dogs as a model of long-segment tracheal reconstruction and transplantation has not met with consistent success, largely because of an inability to maintain tissue vascularity, which compromised short- and long-term survival. In this study, preservation of a previously unrecognized but clearly critical component of...
the transplanted long tracheal segment, the PTF, offered a new solution to the problem. This highly vascular soft tissue “blanket” distributes blood, in a segmental fashion, to an extremely long portion of the trachea from cranially located vessels associated with the thyroid gland complex. Furthermore, the use of large-bodied (>25-kg) mixed-breed animals, vs the previously often used smaller-bodied (~10-kg) purebred beagles, will allow for easier and more reliable microvascular anastomosis of the considerably larger vessels in future studies. In fact, studies are currently under way, first to completely remove the tracheal segment and replace it in the same dog using unilateral arterovenous microvascular anastomoses, then to apply this new model to transplant long segments of trachea across pairs of large-bodied sibling mixed-breed dogs.

Application of tracheal transplantation to humans with long-term long-segment tracheal stenosis would resolve a long-standing clinical dilemma and provide affected individuals with considerable improvement in their quality of life. Establishment of robust techniques in a similarly sized animal model of tracheal transplantation may help us understand better approaches to a problematic and controversial direction in head and neck reconstructive surgery. The need for long-term immunosuppression in such patients represents a negative component of this proposed potential surgical option. However, when this factor is weighed against a markedly improved quality of life, it seems to represent a worthwhile trade-off to correct this debilitating and extremely unpleasant long-term condition.

Accepted for publication January 26, 1999.

This study was supported by a grant from the American Laryngological Foundation and the Department of Otolaryngology, Mount Sinai School of Medicine, New York, NY. We thank Moshe Shalev, MSc, VMD, Russell Jenkins, and Gladys Volmar from the Center for Laboratory Animal Sciences at Mount Sinai School of Medicine for veterinary, operating room, and animal care support, respectively, and for ensuring that the quality of life for the animals used in this study was as pleasant as possible under the circumstances.

Reprints: Patrick J. Gannon, PhD, Department of Otolaryngology, Box 1189, Mount Sinai School of Medicine, Fifth Avenue at 100th Street, New York, NY 10029-6574 (e-mail: pgannon@mountsinai.org).

REFERENCES