The Laryngoscope
Lippincott-Raven Publishers, Philadelphia

Extended Canine Laryngeal Preservation for Transplantation

Kevin F. Kevorkian, MD; Joel A. Sercarz, MD; Ming Ye, MD; Young-Mo Kim, MD; Ki-Hwan Hong, MD; Gerald S. Berke, MD

The goal of successfully transplanting the larynx has motivated researchers since the 1960s. Early laryngeal transplant techniques limited the donor larynx to 45 minutes of ischemia. In this study, a method of prolonged laryngeal preservation is employed in three canines. In vivo cold laryngeal perfusion with University of Wisconsin Solution (UWS) was performed. The larynx was removed and placed into cold storage in 4°C UWS. After 24 hours of storage, the same canines underwent laryngeal reimplantation. The animals were sacrificed 7 days after reimplantation. No evidence of necrosis or vascular insufficiency was identified histologically. The results indicate that canine larynges can be successfully reimplanted after 24 hours of preservation. Future studies will assess the application of this technique to laryngeal transplantation.

***Laryngoscope,* 107:1623–1626, 1997**

INTRODUCTION

Laryngeal transplantation was first studied during the 1960s with the pioneering canine experiments by Ogura, Silver, and Boles.[1–5] This research has been continued in several centers, including the University of California, Los Angeles (UCLA).[6] During our preliminary transplantation research at UCLA, the ischemia time of the donor organ was limited to 30 minutes. Earlier studies by Takenouchi[7] had stated that the larynx can tolerate only 45 minutes of warm ischemic time. The goal of the current study is to develop a technique for cold preservation of the canine larynx to allow a longer ischemic time. If this technique can be applied to the human larynx, procurement of donor organs from an expanded geographic range will be possible.

Principles of organ preservation developed for the heart, liver, and kidney have been applied to the larynx in this study. The University of Wisconsin Solution (UWS) was developed for solid organ transplantation and has been found to prevent ischemia and acidosis in the preserved organs. For example, the acceptable ischemia time for the liver is 24 hours using cold perfusion with the UWS. This proven solution was therefore chosen for initial experiments in laryngeal preservation.

MATERIALS AND METHODS

Harvest Technique

Three mongrel canines each weighing approximately 25 kg were used in this study. Intravenous pentothal was administered until corneal anesthesia was established and the canine was placed in the supine position and intubated orally. Halothane was employed thereafter to maintain the level of anesthesia.

An incision was made in the midline extending from the hyoid bone to the sternal notch. A tracheotomy was performed and an endotracheal tube was inserted into the trachea for ventilation. The hyoid venous arch was dissected bilaterally with care to leave intact the tissue overlying the middle portion of the hyoid venous arch. The cephalic tributaries to the hyoid venous arch were ligated. The common carotid artery and the cranial thyroid artery were then dissected bilaterally.

Presented at the Meeting of the Western Section of the American Laryngological, Rhinological and Otological Society, Scottsdale, Arizona, January 15, 1995.

From the Division of Head and Neck Surgery (K.F.K., M.Y., Y.-M.K., K.-H.H., G.S.B.), UCLA School of Medicine, Los Angeles, and the Division of Head and Neck Surgery (J.A.S.), Harbor UCLA Medical Center, Torrance, California.

This research was supported by the VA Merit Review Funds and by National Institutes of Health grant #RO1 DC 00855-01.

This study was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.). The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles, California.

Send Correspondence to Gerald S. Berke, MD, Division of Head and Neck Surgery, UCLA School of Medicine, 10833 Le Conte Avenue, Los Angeles, CA 90024-1624, U.S.A.

Harvesting of the larynx was undertaken using mucosa-preserving hypopharyngeal cuts. Next, the strap muscles were transected. The external jugular veins were obstructed with microvascular clamps bilaterally and both internal carotid arteries were clamped and ligated. Thirty-five milliliters of 4°C UWS were perfused through the larynx via both carotid arteries. The infusion was continued until the efflux from the larynx was clear. Next, the larynx was removed sharply and placed in a sterile container filled with approximately 250 cubic centimeters of 4°C UWS. The container was wrapped in sterile plastic bags and placed in 0°C cold storage.

The wound was irrigated with saline solution and the pharyngeal mucosa closed. The tracheotomy was converted into a midline tracheostomy and the skin was closed with a penrose drain in the subcutaneous space.

Reimplantation Method

Reimplantation of the cold perfused larynx began 22 hours after the larynx was devascularized. The canine was anesthetized as described above and the neck re-explored. The carotid arteries and the jugular veins ligated previously were identified. The preserved larynx was removed form refrigeration and reanastomosis of both external jugular veins performed with 7-0 Prolene sutures (Ethicon, Inc., Somerville, NJ). The common carotid artery on one side was reanastomosed to reestablish blood flow to the larynx (Fig. 1).

Two of the three canines had only one carotid artery reanastomosed and one had bilateral carotid arteries reanastomosed. The pharyngeal mucosa was closed and the distal end of the larynx was brought out laterally, opposite the tracheostomy, to prevent aspiration. The wound site was irrigated with sterile saline solution and two suction drains were placed. Total laryngeal ischemia time for each canine was 24 hours.

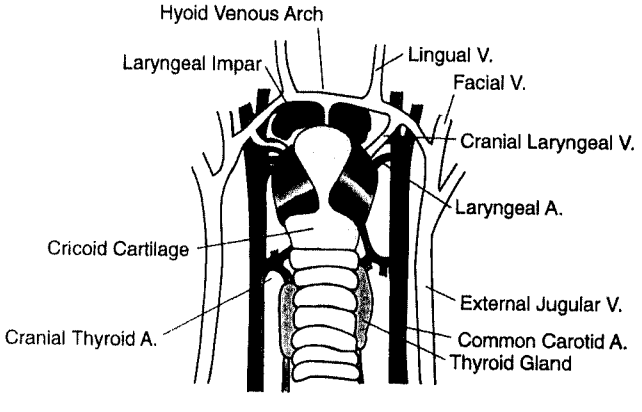

Fig. 1. The canine vascular anatomy demonstrating the locations of the arterial and venous anastomoses for laryngeal reimplantation.

The animals were hydrated intravenously for 1 week. Weight, rectal temperature, laryngeal mucosa appearance, and general physical condition were recorded twice daily. On postoperative day 7, the canine was sacrificed. The reimplanted larynx was removed and preserved in formalin solution for histologic evaluation.

Each animal underwent bilateral reconstitution of the hyoid venous system to the external jugular vein. One animal underwent bilateral arterial anastomosis; the other two animals had unilateral arterial anastomosis.

Close postoperative monitoring demonstrated mild wound infections at the neck incision site in each animal. There was no clinical evidence of sepsis. Wound infections were managed by opening portions of the wounds to facilitate drainage and irrigation. All infections cleared by the 4th day after reimplantation.

On the 7th postoperative day, the larynx was harvested and the animal sacrificed. The larynx was immediately cut in the midsagittal plane and examined for areas of gross ischemia and necrosis. The larynges were cross-sectioned at random multiple sites and stained with hematoxylin and eosin.

RESULTS

During the postoperative period, no apparent ischemia was identified in any of the three canines. At the end of the 7-day survival period, the larynx was examined and appeared viable and pink in color in each case.

When the animals were sacrificed, none of the larynges demonstrated evidence of gross vascular insufficiency or tissue injury. Tissue color and consistency appeared normal in all specimens. Each vascular anastomosis was examined and all arteries and veins with suture anastomoses were bivalved to view the endothelial lining. Each vessel was inspected from the area of the suture anastomosis until entry into the laryngeal tissue. No evidence of gross vascular damage or thrombosis was noted. The entire laryngeal specimen was then immersed in formalin solution for histological evaluation.

Histologic review of all specimens demonstrated normal viable tissue without any ischemic changes, necrosis, or vascular injury (Fig. 2). There was no inflammatory infiltrate noted in one canine and a mild nonspecific inflammatory response in the other two.

Atrophy of the minor salivary glands was present in each case, consistent with the neurogenic atrophy present following transection of the laryngeal nerves. All specimens demonstrated normal-appearing muscular, cartilaginous, vascular, and mucosal cells.

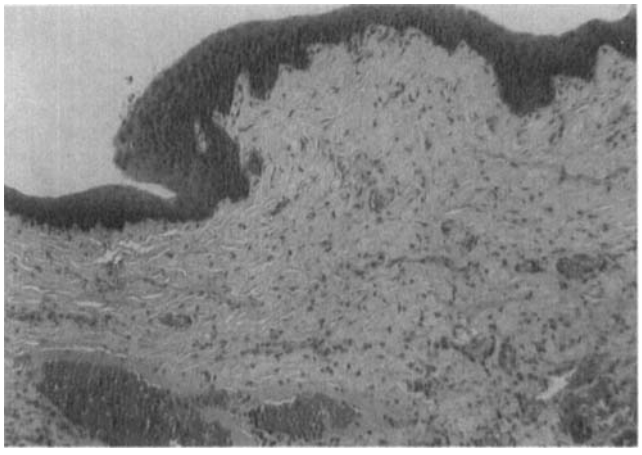

Fig. 2. Laryngeal histology 7 days after reimplantation after 24-hour preservation. The mucosal lining is intact and there is no evidence of cellular or vascular injury (hematoxylin and eosin stain).

DISCUSSION

If human laryngeal transplantation is to become a reality, it may be necessary to transport the donor larynx to distant geographical sites. Because the techniques of cardiac, liver, and kidney transplantation are relatively advanced, considerable research into the preservation of these organs has been completed. Effective methods of organ preservation have proven to increase donor organ viability and significantly extend the possible ischemia time by preventing cell injury.

Recent research into techniques of preservation for solid organs has demonstrated that cold perfusion and cold storage with UWS is an effective method of preservation.[8–10] Strome et al.[8] recently published a study comparing rat larynges preserved with iced heparinized saline to those preserved in UWS. Using UWS, they successfully preserved rat larynges up to 20 hours and examined them clinically and histologically 24 hours after reimplantation. When iced heparinized saline was employed for preservation, all specimens preserved for 6 hours were nonviable. The study supported the use of UWS for laryngeal preservation.[8]

It is encouraging that the canine larynx is capable of being preserved for 24 hours without signs of necrosis 1 week after reimplantation. The fact that each of the larynges was viable 7 days after preservation is important because evaluation of a preserved organ 24 hours after reimplantation may not allow enough time for ischemic changes to occur.

Cold UWS perfusion and cold storage are important for several reasons. First, maintaining proper hypothermia of the organ is necessary for a successful preservation. This is accomplished by storing the UWS and the larynx between 0°C and 4°C at all times. Hypothermia significantly decreases the rate of cellular degradation by intracellular enzymes.[10] Second, flushing the cold UWS throughout the organ thoroughly is necessary to wash out all residual blood products from the vascular channels. This greatly reduces the chance for vascular thrombosis upon reestablishment of vascularity after reimplantation. Third, the preservation solution prevents cell swelling by including components that are osmotically active and impermeable to the cell.[11] Finally, the solution contains hydrogen ion buffers to prevent cellular acidosis and death.

Although laryngeal tissue viability was demonstrated in the study, it is not clear that reinnervation of the larynx would be successful after preservation. In the future, this laryngeal preservation protocol will be tested more fully by performing a transplantation and then assessing the neurologic function of the larynx.

The preservation time of 24 hours used in this experiment most likely exceeds the time necessary to harvest, transport, and revascularize the larynx in potential human applications of laryngeal transplantation. Therefore, when this preservation method is applied to canine laryngeal transplantation, a cold ischemic time of 6 to 12 hours will be substituted.

CONCLUSION

The science of organ transplantation has made significant progress in recent years. Successful organ preservation is a critical part of modern transplantation techniques for the heart, liver, and kidney. With the improved organ preservation, the urgency of the donor-recipient organ exchange is reduced, increasing the size of the donor pool. When cold perfusion and cold storage techniques are applied to the canine larynx, this study demonstrates that the larynx can withstand 24-hour ischemia and remain viable 7 days after reimplantation. Along with advances in microsurgery, immunosuppression, and selective reinnervation, extended laryngeal preservation brings the possibility of laryngeal transplantation a step closer.

ACKNOWLEDGMENT

We would like to acknowledge Du Pont Merck Pharmaceutical Company for their contribution of UWS and Dr. Yao-Shi Fu of the UCLA Pathology Department for reviewing the histology.

BIBLIOGRAPHY

1. Ogura JH, Harvey JE, Mogi G, et al. Further experimental observations of transplantation of the canine larynx. *Laryngoscope* 1970;80:1231–43.
2. Yagi M, Ogura JH, Kawasaki M, et al. Physiological studies of the replanted canine larynx. *Ann Otol Rhinol Laryngol* 1966;75:849–64.

3. Silver CE, Rosen RG. Function of transplanted and denervated larynges. II. Arytenoidectomy with sensory reinnervation. *Arch Otolaryngol* 1974;99:100–1.
4. Silver CE, Rosen RG, Dardik I, et al. Transplantation of the canine larynx. *Ann Surg* 1970;172:142–50.
5. Boles R. Surgical replantation of the larynx in dogs: a progress report. *Laryngoscope* 1966;76:1057–67.
6. Berke GS, Ye M, Block RM, et al. Orthotopic laryngeal transplantation: is it time? *Laryngoscope* 1993;103:857–64.
7. Takenouchi S, Ogura JH, Kawasaki M, et al. Autogenous transplantation of the canine larynx. *Laryngoscope* 1967; 77:1644–67.
8. Strome M, Wu J, Strome S, et al. A Comparison of preservation techniques in a vascularized rat laryngeal transplant model. *Laryngoscope* 1994;104:666–8.
9. Bruhren V, Marzi I, Walcher F, et al. Effects of Euro-Collins, University of Wisconsin, and histidine-tryphtophane-ketoglutarate solution on hepatic microcirculation following liver transplantation in the rat. *Transplant Proc* 1991;23:643–4.
10. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988;45:673–6.
11. Ploeg RJ, Goossens D, McAnulaty JF, et al. Successful 72 hour cold storage preservation of dog kidneys with UW solution. *Transplantation* 1988;46:191–6.