RELIABLE CRYOPRESERVATION OF TRACHEA FOR ONE MONTH IN A NEW TREHALOSE SOLUTION

We previously reported that trehalose, a reduced disaccharide, was effective in the preservation of lungs. In this study, we investigated the possibility of prolonged cryopreservation of tracheas in a preservative solution containing trehalose. Five rings of cervical trachea were removed and immersed in the preservative solution. The harvested tracheas were then cryopreserved and stored in a deep freezer at −85° C. One month later, five rings of mediastinal trachea were removed. The cryopreserved cervical tracheas were thawed and autotransplanted in place of the excised mediastinal trachea ($n = 6$). The anastomotic site and graft were then covered with an omental pedicle. All six animals survived for more than 6 months. All grafts survived without any evidence of atrophy or stenosis. Microscopic examination of the grafts showed that the integrity of the tracheal tissues was maintained. Our findings show that consistent cryopreservation of the trachea for 1 month is possible in a preservative solution containing trehalose. (J THORAC CARDIOVASC SURG 1995;110:382-5)

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The problem of the shortage of organ donors must be solved before tracheal transplantation becomes applicable in the clinical setting. Experimentally, preservation of the trachea has been reported to be possible for about 20 hours by simple immersion in Euro-Collins (EC) solution.[1] The trachea consists of connective tissue, cartilage, and epithelium. Because the structure of the trachea is relatively simple, the possibility of prolonged cryopreservation has been suggested.[2] Cryopreservation of cardiac valves[3] and cartilage[4, 5] has already been successful clinically. The shortage of donors can be resolved if prolonged cryopreservation of the trachea becomes possible.

Trehalose is a nonreducing disaccharide that protects the cellular membrane during cryopreservation.[6-10] We previously reported the effectiveness of trehalose in the preservation of lungs.[11] Others have reported that the combination of membrane-penetrating cryoprotective agents (glycerol, dimethyl sulfoxide [DMSO]) and nonpenetrating cryoprotective agents (trehalose) has a synergistic effect on cryopreserved embryos and spermatozoa.[9, 10]

In this study, we were able to demonstrate the biologic feasibility of prolonged cryopreservation of the trachea with use of a preservation solution containing trehalose and DMSO.

Material and methods

Animals and anesthesia. Six adult mongrel dogs were used in the experiments. Anesthesia was induced by the intramuscular injection of ketamine (10 mg/kg) and the intravenous injection of sodium thiopental (15 mg/kg). After oral endotracheal intubation, the lungs were ventilated mechanically with a Harvard pump (Harvard Apparatus Co., Inc., S. Natick, Mass.) at a tidal volume of 20 ml/kg and a frequency of 20 breaths/min. Anesthesia was maintained with 50% oxygen, 50% nitrogen monoxide, and 1% halothane.

Harvest of grafts. Five rings of the cervical trachea were removed, and the tracheas were anastomosed end to end. The excised section of trachea was simply immersed in a preservative solution that contained Dulbecco's modified Eagle medium, 10% DMSO, 20% fetal calf serum, and 0.1 mol/L trehalose. The grafts were cryopreserved and stored in a deep freezer at −85° C.

Autotransplantation of trachea. After 1 month, the cryopreserved trachea was thawed and autotransplanted to the mediastinal trachea. The graft was thawed at 37° C in an incubator and rinsed with physiologic saline solution 10 times. Tracheal transplantation was done by the technique described previously.[1] Briefly, five rings of mediastinal trachea were removed after the preparation of an

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Table I. *Results of the present study*

Animal no.	*Survival (days)*	*Status of grafts*		*Pathologic findings of grafts (epithelium, cartilage)*
		Shrinkage	*Malacia*	
c-1	200, killed	Negative	Negative	Both preserved
c-2	303, alive	Negative	Negative	—
c-3	296, alive	Negative	Negative	—
c-4	296, alive	Negative	Negative	—
c-5	184, killed	Negative	Negative	Both preserved
c-6	185, killed	Negative	Negative	Both preserved

omental pedicle graft and a thawed tracheal autograft was attached with 4-0 Prolene polypropylene continuous sutures (Ethicon Inc., Somerville, N.J.). The anastomotic site and graft were covered with the omental pedicle graft. Intramuscular administration of cefazolin (1 gm) was continued daily for 1 week after transplantation.

Follow-up bronchoscopic and histologic examination. The graft was examined by bronchoscopy 1, 2, 3, and 4 weeks after operation. Thereafter, bronchoscopic examination was conducted every month. Tracheal tissue was examined histologically when the experimental animals either died or were killed.

The experimental dogs were treated with humane care according to the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23).

Results

Surviving animals (Table I). All animals ($n = 6$) survived for more than 6 months. Three of the animals were killed on postoperative day 184, 185, and 200, respectively, for histologic evaluation. The remaining three animals are still alive 296, 296, and 303 days after transplantation.

Macroscopic and gross findings. The grafts were edematous 1 week after operation. Both the membranous and cartilagenous portions appeared normal and graft edema had vanished completely by 1 month after operation. Endoscopic examination done 6 months after operation revealed no stenosis either in the anastomosis or in the grafts themselves; none of the grafts was atrophic (Fig. 1 and Fig. 2, *A* and *B*).

Histologic evaluation. Normal epithelium covered the graft surfaces, and the viability of the tracheal cartilages was maintained (Fig. 2, *C*).

Discussion

Many problems with regard to the clinical application of tracheal transplantation remain unsolved: immunosuppression,[1, 12] blood supply to the grafts, extent of graft resection,[13] and techniques of graft preservation. In this study, we evaluated a method of graft preservation.

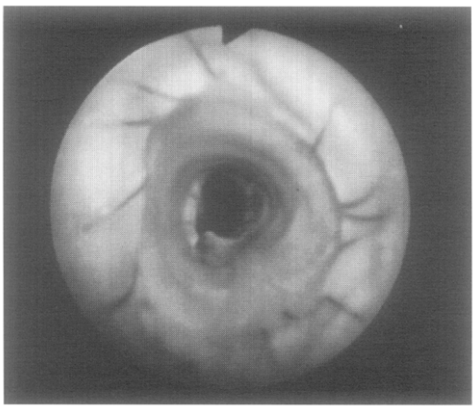

Fig. 1. Bronchoscopic findings 6 months after transplantation. There is no stenosis at the anastomotic site or in the graft. There is no evidence of atrophy in the graft. The graft is covered with normal epithelium.

It is difficult to prepare quickly a graft that is both fresh and appropriate for the patient every time a transplantation is required. We have already reported that tracheas can be preserved for 20 hours by simple immersion in EC solution.[1] It is highly likely that preservation of tracheas is possible for a few days with this technique, but it remains unknown whether preservation over a few months is possible with simple immersion in EC solution.

The trachea is an organ with a simple structure. It consists of tracheal cartilage, mucus membrane, and connective tissues. For this reason, prolonged cryopreservation of the trachea has been suggested previously.[2] Deschamps and colleagues[2] were successful in preserving tracheas for 1 week with mainly DMSO, a conventional cryoprotectant. However, their experimental findings are not sufficient to prove that their method is applicable for clinical transplantation, because their experiments consisted only of heterotopic grafts to the muscular pocket, which are not real transplantations. Moreover, the duration of successful preservation was relatively short in their cases (1 week).

We performed orthotopic autotransplantations after 1 month of cryopreservation. The grafts survived in all six animals in which the tracheal grafts were transplanted. Stenosis and dehiscence of the anastomotic site, graft necrosis, and atrophy were not observed. We believe that our technique of tracheal preservation is a near-perfect system. Our procedure, in which the tracheal grafts are stored

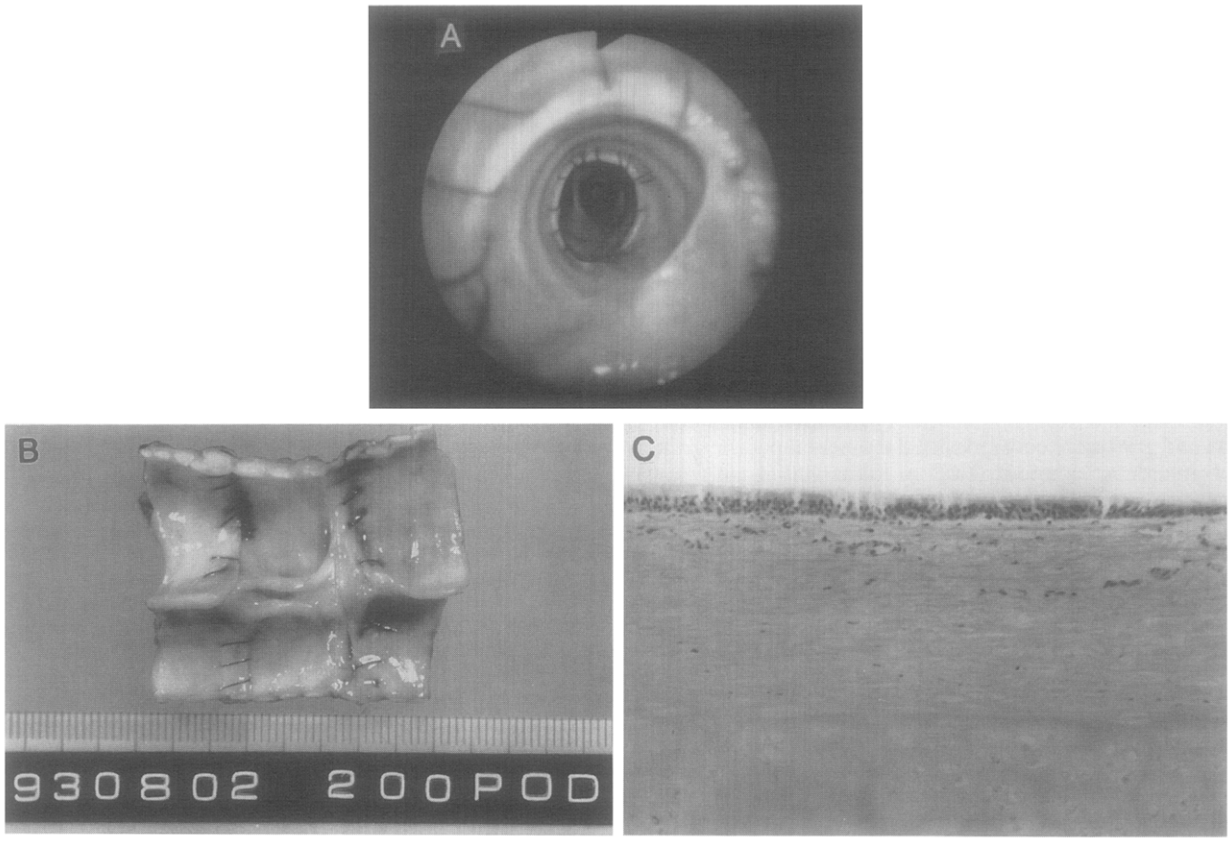

Fig. 2. A and **B,** Both endoscopy and gross examination show that grafts have taken well. No stenosis is seen at the anastomotic site, and there are no signs of necrosis or atrophy of the grafts. **C,** Histologic findings: normal epithelium covers the surface of the graft, and viability of the tracheal cartilage is maintained (original magnification ×100).

directly in a deep freezer at −85° C without the use of a program freezer, is simple to do and is feasible for application in the clinical setting.

DMSO and glycerol are cryoprotective agents that are used conventionally. It has been suggested that these agents protect cells against freezing by intracellular dehydration.[9, 10, 14] Dehydration is induced by the replacment of intracellular water with a cryoprotectant, which penetrates the cell membrane. On the other hand, trehalose cannot penetrate cell membranes. It may act to maintain the viability of the cell membrane by replacing water molecules that are bound to the membrane phospholipids.[8, 9] There are reports that the concomitant use of these two types of cryoprotective agents has a synergistic effect.[9, 10] These observations led us to undertake the present study. The concentrations of trehalose, DMSO, and fetal calf serum used in our experiments were determined according to the regimens of organ-preserving agents that have been reported to be most effective.[15-17] With respect to a control group, there has been no standard method of tracheal cryopreservation to use in a control group. Therefore we did not have such a group in this study. Although the effectiveness of trehalose itself as a cryoprotective agent and its synergistic effect when used in conjunction with other solutions have not been clarified, our findings show that the preservation solution we used in our experiments is effective.

We demonstrated previously that allotransplantation of the trachea is possible without the administration of immunosuppressive agents if the graft is exposed to preoperative irradiation (100,000 cGy).[1] If irradiated grafts can be preserved for a long time by freezing, then tracheal transplantation can be done readily when required from a permanent stock of these grafts in transplantation centers across the country. We are presently conducting experiments involving the prolonged preservation of irradiated grafts as part of the second step of our investigation.

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