Is Tracheal Transplantation Possible With Cryopreserved Tracheal Allograft and Hyperbaric Oxygen Therapy? An Experimental Study

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Background. **Allografts have achieved prominence for tracheal reconstruction because of their natural physiologic and anatomic structure, which preserves respiratory tract flexibility and lumen patency. The immunomodulatory effects of cryopreservation prevent tracheal allograft rejection. In addition, hyperbaric oxygen therapy (HBOT) accelerates wound healing by promoting epithelization and neovascularization. This experimental study investigated the early and late effects of HBOT on cryopreserved tracheal allografts (CTAs).**

Methods. **The study used 33 outbred Wistar rats weighing 300 to 350 g as allograft transplantation donors and recipients. Among these, 22 recipient rats were randomly assigned to the HBOT (n = 11) and control (n = 11) groups. Rats in the HBOT group were treated with 100% oxygen for 60 minutes at 2.5 atmospheres of absolute pressure for 7 days. Recipient rats in both groups were euthanized at 1 week (n = 5) and 4 weeks (n = 6) after transplantation, defined as the early and late periods, respectively.**

Results. **In the early period, no significant histopathologic differences were observed between groups ($p > 0.05$). However, microscopic evaluation of the control group during the late period showed low epithelization of the CTA. In contrast, microscopic evaluation of the HBOT group during this same period revealed epithelium covering the transplanted CTA lumen. Significant epithelization and vascularization and significantly reduced inflammation and fibrosis were found in the HBOT group compared with the control group ($p < 0.05$).**

Conclusions. **HBOT may be effective in tracheal reconstruction by increasing epithelization and neovascularization after extended tracheal resection. HBOT, therefore, should be considered in CTA transplantation.**

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Segmentary resection and primary anastomosis are performed in most cases of tracheal pathologies. However, current surgical techniques limit resection to approximately half of the adult trachea. Many methods, such as allograft, autograft, prosthetic materials, and tissue engineering, have recently been used to provide permanent respiratory tracts for wider tracheal resections [1–3]. Allografts have come into prominence due to their natural physiologic and anatomic structure, which preserve the flexibility of the respiratory tract and enable lumen patency [1, 2]. In addition, previous studies have shown that the immunomodulatory effects of cryopreservation prevent rejection of tracheal allografts, thus reducing antigenicity [1, 4–6].

Hyperbaric oxygen therapy (HBOT) accelerates wound healing by promoting epithelization and neovascularization [7, 8]. Experimental studies have shown the positive effects of HBOT on healing of the anastomosis line after primary tracheal anastomosis [9, 10]. Recent clinical applications based on these study findings indicate that HBOT promotes complete healing in patients with complications of anastomosis (ie, separation, ischemia, etc) [11–13].

On the basis of experimental and clinical studies of the trachea, we hypothesized that HBOT may be effective in cryopreserved tracheal allografts (CTAs). This experimental study investigated the effects of HBOT on CTAs during early and late periods after transplantation.

Material and Methods

The GATA Haydarpasa Training Hospital Ethics and Animal Use Committee approved the animal experimental protocol in this study.

Experimental Animals and Design

The study used 33 outbred Wistar rats weighing 300 to 350 g as allograft transplantation donors and recipients. Tissue exchange between Wistar rats is considered an allograft because this strain is both outbred and randomly bred [2]. The animals were housed at room temperature

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in a natural day/night cycle and were permitted to eat standard rat chow and drink tap water ad libitum during the experimental period.

Eleven rats served as trachea donors, with each rat providing two segments with 3 rings. Twenty-two rats served as transplantation recipients, with the rats randomly assigned to the HBOT (n = 11) or control (n = 11) groups. Recipient rats in both groups were euthanized 1 week (n = 5) and 4 weeks (n = 6) after transplantation, defined as the early and late periods, respectively.

Donor Trachea Harvesting and Cryopreservation

The rats were euthanized by an intraperitoneal injection of an overdose of pentobarbital and their tracheas removed. Grafts were trimmed into 3 segments, placed in sterile tubes, and immediately immersed in a cryopreservative solution (10% dimethyl sulfoxide, 20% fetal bovine serum, and 0.1 mol/L sucrose in Dulbecco Modified Eagle Medium), and stored at –80°C in 5.0 mL freezing tubes for 6 weeks. Before transplantation, the tracheal segments were thawed in a thermostatic water bath at 37°C for 15 minutes. Histologic examination of fresh and cryopreserved tracheal samples confirmed denudation of the epithelium in the cryopreserved tracheas (Figs 1A and 1B).

Tracheal Allograft Transplantation

Recipient animals were anesthetized by an intraperitoneal administration of 80 mg/kg ketamine hydrochloride and 8 mg/kg xylazine and placed supine. The neck was prepared for an aseptic operation by shaving and cleaning with povidone-iodine. A median incision was made, and the muscles were bluntly dissected to expose the trachea. Simultaneously, the lateral longitudinal pedicles on both sides of the trachea were preserved for allograft blood supply. Three tracheal rings were excised below the thyroid cartilage, beyond the level of fourth tracheal ring. The thawed cryopreserved tracheal allografts were transplanted with 7-0 polypropylene sutures. The membranous trachea was first anastomosed, and the cartilaginous trachea was subsequently anastomosed using interrupted sutures (Fig 1C).

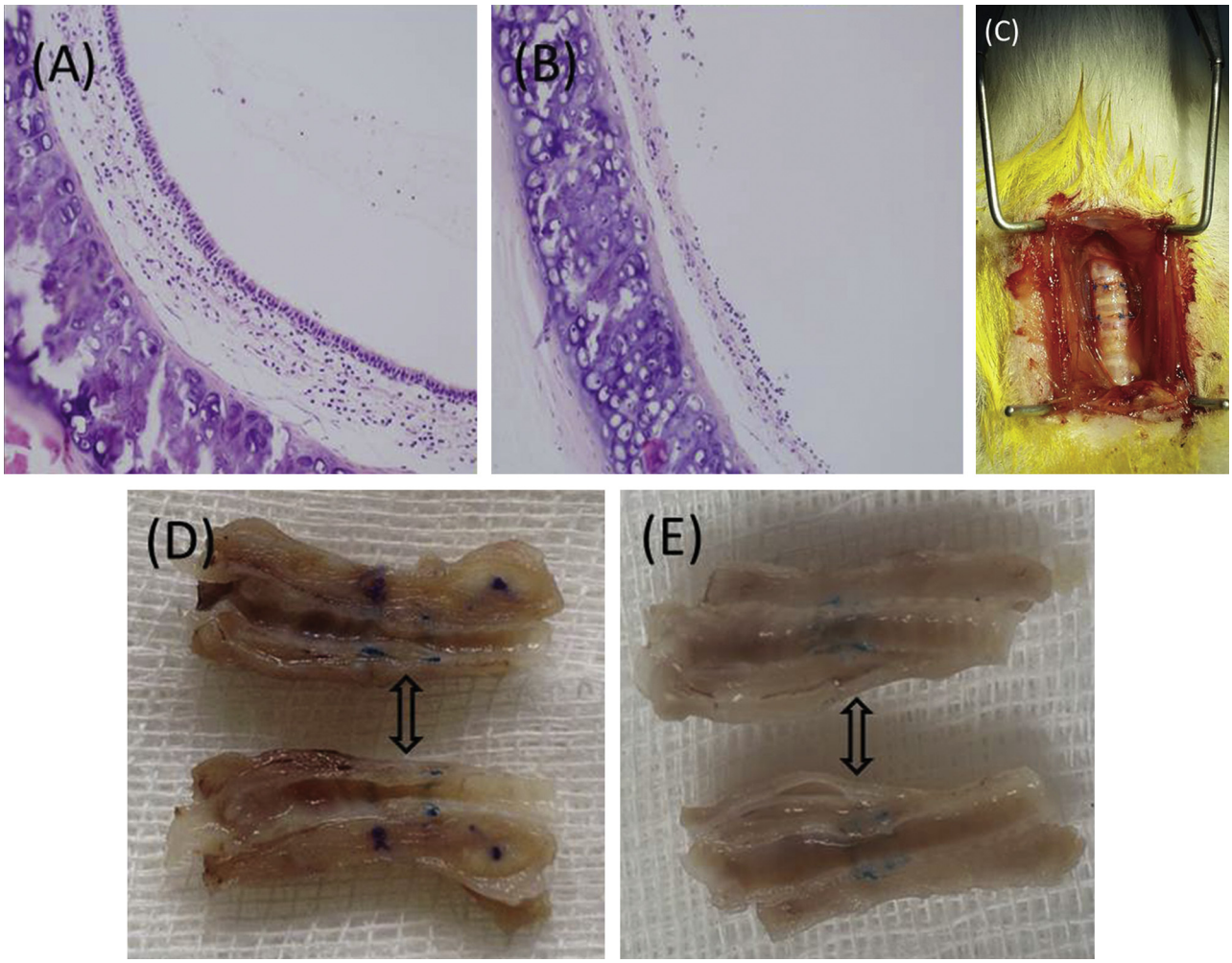

Fig 1. (A) Fresh tracheal epithelium, (B) exfoliated epithelium cryopreserved tracheal allograft (CTA), (C) transplanted CTA in rat showing a macroscopic view of the excised CTA, (D) early period, and (E) late period (hematoxylin and eosin stain; A and B: original magnification × 200).