ESOPHAGEAL REPLACEMENT USING EXTRAINTESTINAL MATERIALS

PhD Thesis

Árpád Juhász M.D.

Unversity of Pécs, Faculty of Medicine

Department of Experimental Surgery

Supervisor:

Professor Erzsébet Rőth

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CONTENTS

1. INTRODUCTION

The pretence to have a safety method for replacement of esophagus is coeval with esophagus-surgery. The results of the surgical treatment of esophageal atresia reveal that, if only a short defect is to be bridged end-to-end repair is possible without the risk of late re-stenosis. After the first successful antethoracal, gastric by-pass of esophagus (Jianu 1903.) several methods have been evaluated to by-pass or after a resection to replace the esophagus; so it means none of them is perfect, according to the late functional results. In the routine surgery stomach, jejunum or the large intestine can be used as a neo-esophagus.

In rare cases, the organ chosen to replace the esophageal defect is too short or unsuitable. During the past 50 years surgeons have long faced the problem of filling such an esophageal hiatus. Various prostheses, allografts and composite grafts have been evaluated, but with no real success to date.

In my work, after the review of esophageal illnesses treated by replacement and its available methods, we have investigated whether the use of a tracheal graft could or could not be used successfully to replace the resected esophagus, and what kind of preparation could create from this organ an available neo-esophagus.

1.1. OVERALL AIM OF THE PROJECTS

- In the first phase of our research we would investigated whether cryopreserved animal trachea is suitable for the replacement of the esophagus. We observed the technical applicability of the operation, survive of animals, suitability of the graft and the postoperative complications.

- In the second phase of our research using MHC-II. (MHC = major histocompatibility complex) antigen specific antibody-staining we have examined whether cryopreservation can reduce the antigenicity of the human trachea and, if so, the optimum duration of preservation.

2. FIRST PHASE OF THE EXPERIMENT: THE ANIMAL MODEL

2.1 INTRODUCTION In this phase we would find a key for the rare cases, the intestine chosen for the replacement of the esophageal defect is too short, and only a couple of centimetres long artificial esophagus would be required in order to create a tensionless anastomosis. To fill the remaining hiatus tracheal allograft would have been used. Before the implantation the graft was cryopreserved to reduce the immunoreaction and to avoid rejection. The examination was based on observations of Japanese researchers, that cryoprecipitation of the trachea may result in the desquamation of inner epithelial layer causing decrease of its antigenity.

2.2 MATERIALS AND METHODS Our experiments were performed in 12 Beagle dogs (mean weight: 10.75 kg) under intramuscular narcosis. Following a median cervical incision, the appropriate muscles were dissected and a 6 cm-long segment of the esophagus was removed, having been cut at 2 cm distal to the pharyngo-esophageal junction. For the replacement of the segment, from other Beagles a 6 cm long tracheal allograft was utilized with a mean internal diameter of 19mm (±1mm). The implant had been cryopreserved before the procedure for 21 days at -86°C. Cooling at a controlled rate was not applied; the samples were transferred into liquid nitrogen in one step. Water bath was used to warm up the cryopreserved tracheal segment to body temperature on the day of the operation. The end-to-end anastomosis was prepared using simple continuous suture (Biosyn® 3/0) and was covered with the sternohyoid flap. The procedure took 116 minutes to perform on average (90-150 minutes). All animals received intraoperative “single-shot” intravenous antibiotic prophylaxis. The dogs only received analgesics (metamizole i.m. 12 and 24 hours following the operation) in the postoperative period; they did not receive

any anti-inflammatory or immunosuppressive agents. All animals were fed parenterally for the first six postoperative days. There was no nasogastric tube inserted. On day 7, oral fluid consumption was initiated. From day 14 they received pasty food, orally. Body temperature was recorded and wound toilette was performed on a daily basis for each animal. Blood samples were collected on the 1st, 3rd, 5th, 7th, 14th, 28th and 56th postoperative days to determine qualitative and quantitative blood counts, including red blood cell count and platelet counts, hematocrit, haemoglobin, size diversity of red blood cells and platelets, plasma fibrinogen concentration and various coagulation parameters. The dogs had been randomised into three groups and were accordingly sacrificed on day 28 (n=4), 42 (n=4) or 56 (n=4). The tracheal graft along with the esophagus and the surrounding tissues was removed for further evaluations. During the air leak test, the esophagus was clamped using an "Akiyama clamp" at 3 cm distal to the graft and a syringe was inserted into the pharynx, - this way apparently closing proximal end – and air was inflated into the submerged graft until it became entirely distended (28-30ml). By measuring the inner diameter of the tracheal lumen, we also calculated the degree of any possible stricture. Furthermore, tissue samples were fixed using 10% neutral formalin and paraffin. Microscopic sections were treated with haematoxylin-eosin staining.

2.3 RESULTS **Survive:** No complications were recorded in the animals during the postoperative period. No suture insufficiency or graft-rejection was observed and the oral feeding of the dogs was free of any complications. In two cases wound suppuration was found involving only the cutaneous and subcutaneous layers. **The graft:** Those grafts, left in place for 56 days shortened from 60mm to a mean length of 47 mm (±3mm). The sutures withstood to the air-probe with excellent results. In 56 days, the mean internal

diameter constricted from 19mm (±1mm) to 15.8mm on average (±0,6mm). **Laboratory results:** Laboratory parameters characteristic for inflammation were altered in accordance with the peri-operative stress: following a temporary increase in the early postoperative period, the measured variables returned to the control value on the 7th postoperative day. On day 14, the same variables were at the base-level. All other examined parameters were essentially unchanged during the study period. **Histology:** Histological examination of the trachea prior to implantation revealed the disintegration of the inner epithelial layer. Histological examination revealed that the esophageal epithelium adhered to the neo-esophagus prepared from the trachea by the fourth week; the native columnar-epithelium of the trachea was not detectable in the graft. Inflammation and granulation were merely revealed in the suture-lines and diminished later on. Inflammation characterized by acute inflammatory cells was present around the tracheal cartilages and the disintegration of these cartilages was also observed.

2.4 DISCUSSION This phase was accompanied by a good result; in dogs the use of cryopreserved trachea for replacement of a 5-6 cm esophageal segment seems to be a promising technique.

3. SECOND PHASE OF THE EXPERIMENT: CRYOPRESERVATION OF THE HUMAN TRACHEA

3.1 INTRODUCTION In this phase of our research we have examined whether cryopreservation can reduce the antigenicity of the human trachea and, if so, the optimum duration of preservation.

3.2 MATERIALS AND METHODS During tracheostomy, segments of the tracheal wall were removed from 50 patients (mean age: 55 years (19-85), 34 males/16 females). These segments, which contained all the tracheal layers, were removed with scissors; bipolar scissors were not used in order to avoid thermic lesions. In this part of the trachea, the intubational cuff was not pumped up, which might have caused compression injuries. The removed segments were free of inflammation or malignancy, as confirmed by histopathological examinations. They were divided into 4 parts. One part was placed in formalin and histopathological examinations were performed immediately (Group 1). The other 3 parts were stored in liquid nitrogen for 2 (Group 2), 3 (Group 3) or 4 weeks (Group 4). Cooling at a controlled rate was not applied; the samples were transferred into liquid nitrogen in one step. After the subsequent warm-up, microscopic sections were subjected to staining with haematoxylin-eosin and MHC-II antigen-specific antibody. During the evaluation, the intensity of staining was graded on a scale of from 0 to 3+. After this grading, the mean intensity of staining and its standard deviation were calculated by using the Student T-test. All experiments were carried out in full accordance with the relevant Hungarian legal and ethical requirements, and all the involved patients gave their signed informed consent.

3.3 RESULTS Four of the 50 trachea segments could not be evaluated histologically, because the preparation did not contain sufficient epithelium for the examination. Accordingly, 46x4 (184) parts from 46 samples were worked up statistically. HE staining demonstrated that there was no desquamation of the respiratory epithelium after cryopreservation. The MHC-II antibodies were mostly situated in the apical part of the epithelial cells. The mean intensity of staining was 1.5+ – 1.9+ with a decreasing tendency, but the intergroup differences were not significant.

3.4 DISCUSSION The cryopreservation of the human trachea does not cause epithelial desquamation from the inner surface. The mean intensity of MHC-II staining decreased not significantly, so independently of its duration this method does not cause a significant reduction in the antigenicity of human trachea.

4. OVERALL DISCUSSION

The aim of our research was to find an applicable method to replace the esophagus by using an extraintestinal tissue.

When segment resection is necessary for the treatment of esophageal diseases, resection and reconstruction are the consecutive steps of the operative plan. Primary reconstruction, with end-to-end anastomosis is only feasible in case of a short defect. In case of a more extensive hiatus, esophageal replacement is the method of choice. In rare cases, the conventional organs (stomach, jejunum, large intestine) for replacement are not feasible. Various methods and materials have been evaluated to bridge this gap, with no real success to date.

We would investigate whether the trachea could be used to replace a short segment of esophagus.

Anatomically, the trachea is an organ with scant blood-supply: its cartilagous portion is avascular, would be an ideal organ for the replacement. Over the last 20 years several researchers have developed experimental models for tracheal replacement using allogenous trachea segments. According to these observations, the transplant receives its nutritive supply mainly from blood vessels emerging from the anastomoses following the implantation. This might serve as the principal reason why the length of the safety implantable trachea segment is limited to 5-6 cm; otherwise necrosis is likely to develop in the middle part of the graft. Confirming the results of the Japanese authors, those factors principally responsible for antigenecity and thus for graft rejection seem to be located on the inner epithelial layer of the trachea. Epithelial desquamation, caused by cryopreservation, may result in the loss of antigenity. In our animal experiments the cryopreservation was used

exclusively; and the trachea transplantation was completed following the cryopreservation of the graft. Histological examination revealed that the graft incorporated normally into the body. According to our data in vivo, the trachea seems to be a feasible organ for the replacement of a 5-6 cm oesophageal segment, and the 21-day-long cryopreservation alone seems to be enough to reduce the antigenecity of the organ and to prevent its rejection.

In the next phase we have examined whether this method could be applicable in the human treatment too and the cryopreservation could reduce the antigenicity of the human trachea. The results were contrary to our expectations, cryopreservation of the human trachea does not cause desquamation of the inner surface and accordingly there is no significant decrease in the intensity of immunological staining. Independently of its duration, cryopreservation alone is not sufficient to allow use of the human trachea as an allograft.

Anatomically the trachea seemed to be available to create a neo-esophagus, so we thought this aim had justification. If the result had been positive, a “trachea-bank” would have been created from cryopreserved organs. But we can conclude that, the cryopreservation of the trachea gave us not the expected result.

In the following research we would investigate the reason of the different immunological behaviour of animal and human trachea. During the cryopreservation we would apply cooling at a controlled rate or cryoprotective agent. After this phase the problem seems to be not surgical, the key can be in the development of immunosuppressive medication, or in the research of stem-cells. Maybe an artificial neo-esophagus will be created from pluripotent cells in the future.

5. NOVEL FINDINGS

- In animal models we have verified that the 21-day-long cryopreservation of the trachea can cause epithelial desquamation on the inner surface, so cryopreservation alone seems to be enough to reduce the antigenecity of the organ and to prevent its rejection.

- In dogs the cryopreservated trachea seems to be a feasible organ for the replacement of a 5-6 cm oesophageal segment, the organ can completely incorporate by the fourth week into the recipient body, the esophageal epithelium adheres to the neo-esophagus.

- Cryopreservation of the human trachea does not cause desquamation of the epithelial layer; this preparation can decrease the antigenecity of the organ, but the tendency is not significant. So we can conclude that cryopreservation alone is not sufficient to allow use of the human trachea as an allograft.

6. PUBLICATIONS, PRESENTATIONS

publications in connection with Thesis

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presentations in connection with Thesis

Árpád Juhász: Esophageal replacement using cryopreserved tracheal graft. *11^{th} World Congress of the International Society for Diseases of the Esophagus, 10-13.Sept 2008 Budapest, Hungary*

Juhász Árpád: A human trachea cryopreservatioja az immunogenitás változásának tükrében. *22th Congress of Experimental Surgery of the Hungarian Surgical Society, 11-13. Jun 2009 Szeged, Hungary*

presentations without connection with Thesis

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