Effects of basic fibroblast growth factor on early revascularization and epithelial regeneration in rabbit tracheal orthotopic transplantation

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Abstract

Objectives: Donor airway ischemia is a significant problem after tracheal replacement with homograft or lung transplantation. Omentopexy is the usual countermeasure to prevent or overcome the ischemia of the airway but this is frequently not sufficient. This study was designed to investigate whether basic fibroblast growth factor (bFGF) can augment tracheal revascularization and its epithelial regeneration in rabbit tracheal autograft. Methods: About half the length (44±45%) of the trachea of New Zealand white rabbit were autotransplanted in the original position immediately after harvest. In group I (n = 15, control group), cervical tracheal autotransplantation was done only. In group II (n = 15, omentopexy group), the cervical tracheal autograft was wrapped with subcutaneously advanced omentum. In group III (n = 15, bFGF group), 1 μg of bFGF was applied evenly on the graft after the completion of anastomosis. Five animals in each group were examined on the 3rd, 7th and 14th postoperative days. Three rings of trachea were taken at the mid portion of the graft and the supra-carinal untouched normal trachea in each. The effect of revascularization was assessed by measuring the uptake of human serum albumin labeled with 99m technetium, which was injected into the left atrium just before sacrifice. The epithelial regeneration was assessed by means of light microscopic examination. Results: The proportion of perfusion of the graft to normal trachea was much higher in group III (P < 0.05) on day 3 (25.4, 27.8 and 54.7% in groups I, II and III, respectively), but there was no difference on the 7th and 14th days. The epithelial regeneration was better in group III (P < 0.05) than in the other groups on day 3, and was better in groups II and III than group I on day 7. Conclusion: We concluded that bFGF enhances the revascularization and epithelial regeneration of the tracheal autograft, especially during their early phases.

Keywords: Basic fibroblast growth factor; Revascularization; Epithelial regeneration; Tracheal transplantation

1. Introduction

Tracheal resection and end-to-end anastomosis is the most common method in tracheal surgery. However, this is not feasible when the disease involves long segments of the trachea. To solve this problem, implanting prosthetic materials or tissue grafts were attempted, but these methods were not successful because of their undesirable disadvantages such as rigidity, frequent granuloma formation, frequent migration from the original site, damage to the adjacent vessels by the prosthetic materials, and inability to maintain the original round shape of the trachea, because of ischemic degeneration of implanted trachea [1–5].

Trachea homograft seemed a good alternative but also had weak points such as rejection and ischemia [6–10]. The revascularization is achieved from adjacent tissue and trachea and usually takes 12–15 days. Consequently during the initial post-transplantation period, the graft can remains in the ischemic condition that could result in necrosis, stenosis and dehiscence of the graft.

Several methods were studied to prevent ischemia and promote local revascularization. The most potent method was wrapping of the graft with advanced omentum, but this still took 4–5 days for promoting revascularization [11,12]. Hence the other methods to improve early revascularization within 4 days are required. PGI2, heparin and many various growth factors are known to promote revascularization in the acute phase. Among these agents, basic fibroblast growth factor (bFGF) has attracted attention because of its potent ability to enhance revascularization, although these are some controversies about its effects [13–15]. Even in reports supporting the effective influence on revascularization, there were some problems such that it was not possible to rule out the influence of the omentum.
and other abdominal factors because the graft was implanted in the abdominal cavity.

To evaluate the effect of bFGF precisely in improving early revascularization, this study employed the orthotopic cervical autograft method. We avoided tracheostomy and tracheal intubation, because these procedures might damage the tracheal graft. This study attempted to shed light on the effects of bFGF on revascularization and obtain some understanding of its potential in clinical use.

2. Materials and methods

2.1. Animals

Forty-five adult white New Zealand rabbits weighing from 2.0 to 3.0 kg were premedicated and anesthetized with an intramuscular injection of 50 mg/kg ketamine hydrochloride and 2 mg/kg xylazine. They were placed in the supine position and draped to expose the neck and abdomen. Anesthesia was maintained well with the above dosage and sometimes an additional dose of xylazine (1 mg/kg) was given. Self-respiration without mechanical ventilation was maintained during the entire operation. All animals received human care in compliance with the European Convention on Animal Care and this study was approved by the Ethics Committee of our hospital.

2.2. Surgical technique and management of autograft

A longitudinal neck incision was performed under sterile conditions, and the cervical trachea was exposed. A segment about 12 rings of the trachea was excised and rinsed in sterile saline for approximately 2 min. This segment represented about half the length of the entire trachea of the rabbit. The excised trachea was then reimplanted in its original position as an autograft, with the same orientation. The lower anastomosis was done first with continuous 6-0 prolene polypropylene sutures (Ethicon Inc., Sommerville, NJ) and then proximal upper anastomosis was done subsequently in the same fashion.

In one group, the animals underwent omental wrapping after reimplantation of the autograft. After midline laparotomy, an omental pedicle was processed without splenectomy. The right gastroepiploic artery fed the omentum. The omental pedicle was brought up into the neck via the right-sided parasternal subcutaneous route. This procedure was important, since the parasternal route prevents injury to the omentum during later median sternotomy for evaluation of revascularization. We checked omental viability in terms of its color and arterial pulsation just before wrapping the trachea. All omental pedicles maintained good viability as a result. The whole length of the autograft was wrapped with omentum circumferentially and secured with a few sutures at both ends. The neck and abdominal incisions were then closed in standard fashion. The total operating time was approximately 20–30 min depending on additional procedures. All animals received antibiotics intramuscularly for the first 2 days.

2.3. Grouping

The 45 animals were randomly assigned to one of three treatment groups (n = 15 for each group). In group I (control), the tracheal autograft was simply re-implanted in its original position. In group II (omentopexy), the tracheal autograft was re-implanted in the same way as group I and wrapped with omentum. In group III (bFGF), 1 µg of basic fibroblast growth factor (recombinant bFGF from Escherichia coli, Boehringer Mannheim, Germany, diluted to 2 µg/cc with phosphate-buffered saline) was applied evenly to the outer surface of autograft after reimplantation into the original position in the same way as group I (Fig. 1).

2.4. Assessments

Three, 7 and 14 days postoperatively, five animals were anesthetized in an identical method and maintained under spontaneous respiration. We did not intubate the trachea and did not perform tracheostomy so as to avoid possible damage to the tracheal graft. Midline sternotomy was performed carefully with attention not to open the pleural cavity and not to injure the omental pedicle, because bilateral pleural tear could impinge upon ventilation and produce hypoxia.

2.4.1. Assessment of microvasculature

After opening the pericardium vertically, the left atrial appendage was exposed and 3000 IE heparin was given via the appendage; thereafter 1 ml human serum albumin labeled with 99m technetium (300–500 µCi/ml) was injected. After 2 min of circulation the animals were killed and the neck was opened. The adjacent tissue was carefully removed and the whole length of the trachea from the cricoid cartilage to the carinal bifurcation was exposed. In the omentopexy group, omental tissue was also removed completely and carefully. The entire length of the trachea and grafted tracheal length were measured in situ. The entire trachea was excised, and the radioactivity of the normal trachea and the autograft was measured with a scintillation counter (Packard, USA). A 6–8-mm length of midportion of the grafts and normal untouched portion of the trachea (far from the graft, 0.5 cm above the carina) were excised in each animal for radioactivity and histologic measurement and weighed in milligrams (Fig. 2). Measurement of radioactivity was corrected for weight (counts/min per mg). Activity of the graft was computed as a percentage of activity of the normal tracheal segment.

2.4.2. Histologic examination

The same specimens, midportion of the graft and supra-carinal trachea, were fixed with 10% formalin solution, and stained with hematoxylin and eosin. Under light micro-
scopy, the degree of tracheal epithelial regeneration was evaluated according to the following scale, which was suggested by Mayer [13]: Grade 0, no epithelium and single non-confluent epithelial cells; Grade I, confluent single layer, non-ciliated epithelium; Grade II, confluent multilayer, non-ciliated epithelium; Grade III, normal mucociliary epithelium.

2.5. Statistical analysis

All parametric data were expressed as mean ± 1 standard error of the mean. Statistical analysis was performed with the Wilcoxon rank sum test and Kruskal–Wallis test for parametric data, and Ridit test for non-parametric histopathologic data. The level of significance was defined as $P$ value less than 0.05.

3. Results

3.1. Profiles of animals and grafts

All animals survived to the end of the experiment and did not show any symptoms of airway obstruction. There were no statistically significant differences in body weight between groups (2.5 ± 0.1 kg in group I, 2.5 ± 0.1 kg in group II and 2.4 ± 0.1 kg in group III, $P > 0.05$). Also, there were no differences between groups in length of graft and the proportion of graft length to whole trachea (2.4 ± 0.1 cm and 45 ± 1.1% in group I, 2.3 ± 0.1 cm and 45 ± 1.0% in group II, 2.1 ± 0.1 cm and 44 ± 0.9% in group III, $P > 0.05$) (Table 1). Grossly there was no necrosis, infection or dehiscence of any graft.
3.2. Revascularization

The degree of revascularization of the graft was expressed as the uptake ratio of radioactivity of the graft over normal trachea. The technetium uptake ratio of graft after 3 days was 25.4 ± 6.7% in group I, 27.9 ± 6.0% in group II and 54.7 ± 8.2% in group III. There was a statistically significantly higher technetium uptake in group III compared with groups I and II (p < 0.05). However, there were no differences between the three groups at 7 days and 14 days (Table 2).

3.3. Histologic results

There were no changes in normal tracheal epithelium in any group at any day. At postoperative day 3, control and omentum group showed some sloughing of epithelium and at best grade 1 gradings. The bFGF group (group III) showed better epithelial morphology, however there was no difference between groups I and II. On the 7th postoperative day, the epithelial regeneration score was better in groups II and III compared to group I, and also group III was better than group II (p < 0.05). However, there was no significant difference of epithelial regeneration between the three groups on the 14th postoperative day (Table 3).

4. Discussion

After lung transplantation donor airway ischemia is still a significant problem and result in a graft necrosis and dehiscence. To increase its vascularity many trials have been attempted, and omental wrapping was used widely after lung transplantation several years ago. However, omental wrapping could not completely resolve the ischemia problem, and the need for new agents to improve revascularization in the early phase has been recognized [16].

Fibroblast growth factor has attracted attention by many researchers because of its strong and rapid revascularization effects. In 1989 Olech et al. [14] applied bFGF to rabbit trachea, but could not produce improved revascularization perhaps due to the inadequate dosage of 10 ng. Although 10 ng of bFGF is sufficient in cell culture, a higher dose is required to enhance revascularization in vivo. Mayer et al. [13] and Albes et al. [15] reported successful outcomes with bFGF in tracheal revascularization in 1992 and 1994, respectively. They used a higher dose and a continuous release method of bFGF. However, there were some problems: it was not possible to rule out the influence of the omentum and other factors related to the abdomen especially in the former study, because they implanted the graft in the abdominal cavity.

Our cervical autograft model has several advantages compared with other studies. This model is very convenient, cheap, easily reproducible and easily applicable to the clinical situation. It also avoids the influence of abdominal factors and blind secluded airway problem. This model makes it possible to prevent mechanical graft injury while maintaining of self-respiration, because tracheal intubation, tracheostomy and mechanical ventilation are not required. With this autograft model we were able to avoid problems of rejection that could downsize the effect of revascularization.

For bFGF to be used clinically, the optimal dosage of bFGF and optimal drug delivery method must be determined. Several authors used different doses varying from 10 ng to 2.5 μg. Although Olech et al. [14] failed to improve

<table>
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<tr>
<th>Group</th>
<th>Postoperative day</th>
<th>Percentage of technetium uptake</th>
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<tr>
<td>Group I</td>
<td>3</td>
<td>25.4 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>64.4 ± 4.7</td>
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<td></td>
<td>14</td>
<td>69.6 ± 9.6</td>
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<td>Group II</td>
<td>3</td>
<td>27.8 ± 6.0</td>
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<td></td>
<td>7</td>
<td>62.2 ± 2.7</td>
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<tr>
<td></td>
<td>14</td>
<td>73.4 ± 10.0</td>
</tr>
<tr>
<td>Group III</td>
<td>3</td>
<td>54.7 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>59.2 ± 10.6</td>
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<td></td>
<td>14</td>
<td>75.6 ± 5.3</td>
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Table 2
Percentage of 99mtechnetium uptake of the graft with time in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Postoperative day</th>
<th>Histologic Grade</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
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<td>4</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<td></td>
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<tr>
<td>Group II</td>
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<td>1</td>
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</tr>
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<td></td>
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<tr>
<td>Group III</td>
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early revascularization with 10 ng of bFGF, Mayer et al. reported successful results with 24 ng of bFGF per day for 6 days. Albes et al. [15] also showed improved revascularization using fibrin glue enriched with 2.5 μg of bFGF [13–15]. We chose an intermediate dosage, and succeeded in enhancement of revascularization using 1 μg of bFGF. Higher dosages of bFGF might be eventually harmful to the graft because bFGF enhances growth of fibroblast, which results in granuloma formation, and later stenosis of graft. Our results in rabbits suggest that a dose of around 1 μg bFGF can improve revascularization in tracheal grafts.

The duration and mode of application of bFGF must also be established. It is desirable to infuse the bFGF continuously for 14 days, because it is known that natural revascularization by adjacent local tissue is established after 14–15 days. In this study, revascularization at day 3 in group III was superior to those in groups I or II, but those at days 7 and 14 were not. This kind of short effect may be related to our single administration. Consequently some experiments of continuous application for 14 days would be of interest. Mayer et al. designed an apparatus for continuous application with pump and a small-caliber catheter, while Albes et al. [15] adopted a mixture of fibrin glue with bFGF. However, Mayer’s apparatus is not easy to apply clinically due to the increased risk of infection and the cumbersome indwelling catheter. Although the fibrin glue enriched with bFGF is more practical clinically, the chronological period and mode of release of bFGF from the fibrin glue is unclear and the degree of the angiogenic effect of bFGF is also difficult to evaluate accurately. Moreover, the fibrin glue may increase the chance of infection.

It is known that bFGF enhances not only vascularization but also epithelial proliferation. However, it is not known whether enhanced epithelial regeneration in bFGF-treated trachea is caused by the direct effects of bFGF on epithelium or secondarily by the effects of enhanced revascularization, or both. This question is important in tracheal replacement with homografts and should be answered. If bFGF increases epithelial proliferation of homografts, it would result in an increased degree of rejection because the epithelium of the homograft could play a major role in rejection. Recently, Mukaida et al. noticed that the origin of the cryopreserved tracheal epithelium was outgrowth of the recipient’s epithelium [17]. Based on our studies, we arrived at the conclusion that bFGF enhances revascularization and epithelial regeneration of rabbit tracheal autograft especially during the early post-transplantation phase. We recommend that the optimal dosage and the methods of continuous release of bFGF should be established before being applied clinically.

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References