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Slow release of basic fibroblast growth factor (b-FGF) promotes growth of tracheal cartilage

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Abstract
Purpose: Tracheomalacia is a major cause of morbidity in conditions such as oesophageal atresia. However, symptoms usually improve with age. A more rapid growth of tracheal cartilage can be induced by basic-Fibroblast Growth Factor (b-FGF). This study aimed to investigate whether slow-release b-FGF could act as a novel treatment for tracheomalacia.

Methods: Biodegradable gelatin hydrogel sheets incorporating 0.5, 5, or 50 μg/20 μl of b-FGF solution were inserted between the cervical trachea and esophagus of rats. No intervention was performed in rats in a control group. All animals were sacrificed 4 weeks later, and the luminal area of the cervical trachea and the thickness of the cartilage were measured.

Results: The mean luminal areas in the control group and in the b-FGF groups were 3.1, 3.2, 3.8, and 2.6 mm², respectively, and showed a peak area at 5 μg of b-FGF. A significant difference was seen only between the control group and the b-FGF 5 μg group (p<0.05). The mean thickness of the tracheal cartilage was 0.12, 0.13, 0.19, and 0.32 mm in the control and the b-FGF groups, respectively, and showed a dose-dependent increase, which was statistically significant between the b-FGF 5 μg or 50 μg groups and the control group (p<0.01).

Conclusion: This study showed that slow-release b-FGF enlarges the tracheal lumen and thickens the cartilage in a dose-dependent fashion.

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Treatment for severe tracheomalacia is controversial [1,2]. Traditionally, symptoms in children with moderate to mild tracheomalacia are thought to improve as they grow older [3–6] and we have case-based experience where the patients’ tracheostomy tubes can be extubated later in childhood without ill effect. This phenomenon is believed to be caused by a growth-related increase in the size of the trachea.

Basic fibroblast growth factor (b-FGF) is a very effective growth factor that induces angiogenesis and wound healing as a result of its action on smooth muscle cells, endothelial cells, fibroblasts, and epithelial cells [7,8] and may also be known as chondrocyte growth factor [9,10]. It rapidly diffuses from the implant site when applied in solution [11–13], and a slow-release version of b-FGF has been developed...
where it is immobilized by acidic gelatin hydrogel through ionic interaction. This technique therefore enables controlled release of b-FGF as a result of hydrogel degradation [14,15]. Several studies have reported that b-FGF enhances cartilage regeneration, and suggested the possibility that a tracheal defect might be repaired using tracheal cartilage engineered by this technique [16–18]. However, to our knowledge, no study has been published that investigates the feasibility of treating tracheomalacia with b-FGF.

The aim of this study was to investigate whether slow-release b-FGF applied externally promotes tracheal growth.

1. Materials and methods

The study protocol was approved by the Animal Care and Use Committee of The University of Tokyo (protocol No. 08-P-93) and all experiments were performed in accordance with Guidelines for Proper Conduct of Animal Experiments of the University of Tokyo.

1.1. Preparation of slow-release forms of b-FGF

b-FGF-impregnated gelatin hydrogel sheets were produced as previously described [14]. Briefly, a 5% aqueous solution of gelatin with an isoelectric point of 5.0 (Nitta Gelatin Co., Osaka, Japan), containing 0.05% by weight glutaraldehyde (Wako Pure Chemical Industries, Osaka, Japan), was cast into a Teflon mold, then stored at 4 °C for 12 h for completion of cross-linking reactions. The material was then immersed in glycine solution at 37 °C for 1 h, washed with distilled water, lyophilized, and sterilized by ethylene oxide. Human recombinant b-FGF (0.5, 5, and 50 μg) (Kaken Pharmaceutical Co. Ltd., Tokyo, Japan) dissolved in 20 μl of distilled water was added to approximately 2 mg of lyophilized gelatin hydrogel sheets, which were cut into pieces of 4 mm in height × 5 mm in width, and kept at room temperature for 30 min before use. This gelatin hydrogel sheet is biodegradable and absorbed in about 2 weeks and releases b-FGF gradually as the sheet is degraded [14].

1.2. Surgical procedure

A total of 32 3-week-old Wistar rats were divided into 4 groups (control, b-FGF 0.5, 5, 50 μg) at the time of placement of the gelatins were 47 (12.6), 40 (2.6), 45 (5.2), and 40 (2.3) g, respectively, with no significant difference between them. The mean body weights (SD) of animals in each groups (control, 0.5, 5, 50 μg) at the time of placement of the gelatins were 47 (12.6), 40 (2.6), 45 (5.2), and 40 (2.3) g, respectively, with no significant difference between them. The mean body weights (SD) at the time of sacrifice were 196 (22.5), 185 (12.3), 187 (8.8), and 175 (7.2) g, respectively. The body weight of rats in the b-FGF 50 μg group was lower than those of the other groups, but otherwise there was no significant difference between them. The mean body weights (SD) at the time of sacrifice were 196 (22.5), 185 (12.3), 187 (8.8), and 175 (7.2) g, respectively. The body weight of rats in the b-FGF 50 μg group was lower than those of the other groups, but otherwise there was no significant difference between them.

The mean body weights (SD) of animals in each groups (control, 0.5, 5, 50 μg) at the time of placement of the gelatins were 47 (12.6), 40 (2.6), 45 (5.2), and 40 (2.3) g, respectively, with no significant difference between them. The mean body weights (SD) at the time of sacrifice were 196 (22.5), 185 (12.3), 187 (8.8), and 175 (7.2) g, respectively. The body weight of rats in the b-FGF 50 μg group was lower than those of the other groups, but otherwise there was no significant difference between them. The mean body weights (SD) at the time of sacrifice were 196 (22.5), 185 (12.3), 187 (8.8), and 175 (7.2) g, respectively. The body weight of rats in the b-FGF 50 μg group was lower than those of the other groups, but otherwise there was no significant difference between them.

Cross-sections of the tracheas in each groups are showed in Fig. 1. The luminal areas of animals in the control group and each of the b-FGF groups (0.5, 5, 50 μg) were 3.1 (0.3), 3.2 (0.3), 3.8 (0.6), and 2.6 (1.3) mm², respectively, and showed a bell-shaped dose–response curve with a peak at 5 μg of b-FGF (Fig. 2). The luminal area of the b-FGF 50 μg group was
the smallest, and the value was smaller than that of the control group. There was a significant difference only between the control group and the b-FGF 5 μg group (P<0.05). The thickness of the tracheal cartilage was 0.12 (0.01), 0.13 (0.01), 0.19 (0.04), 0.32 (0.08) in the control and b-FGF groups, respectively, and showed a dose-dependent increase, which was statistically significant between the b-FGF 5 or 50 groups and the control group (Fig. 3; P<0.01, respectively).

### 3. Discussion

We have shown that slow-release b-FGF promoted growth of the adjacent trachea. This effect was dose-dependent for tracheal thickness but appeared to be a bell-shaped dose–response effect with a peak at 5 μg of b-FGF for the luminal area. It means that there is an optimal dose of b-FGF that has a maximal effect on tracheal cartilage growth.

### Table 1 Results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Weight at the operation (g)</th>
<th>Weight at the sacrifice (g)</th>
<th>Luminal area (mm²)</th>
<th>Thickness of the cartilage (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>47 (12.6)</td>
<td>196 (22.5)</td>
<td>3.1 (0.3)</td>
<td>0.12 (0.01)</td>
</tr>
<tr>
<td>FGF 0.5</td>
<td>6</td>
<td>40 (2.6)</td>
<td>185 (12.3)</td>
<td>3.2 (0.3)</td>
<td>0.13 (0.01)</td>
</tr>
<tr>
<td>FGF 5</td>
<td>8</td>
<td>45 (5.2)</td>
<td>187 (8.8)</td>
<td>3.8 (0.6)*</td>
<td>0.19 (0.04)**</td>
</tr>
<tr>
<td>FGF 50</td>
<td>6</td>
<td>40 (2.3)</td>
<td>175 (7.2)</td>
<td>2.6 (1.3)</td>
<td>0.32 (0.08)**</td>
</tr>
</tbody>
</table>

Data are shown as mean (SD).

* P<0.05.

** P<0.01.

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**Fig. 1** Cross-section of the trachea in each group. The luminal area of the b-FGF 5 μg group is the largest and that of the b-FGF 50 μg group is the smallest of all groups. The thickness of the tracheal cartilage increases dose-dependently.
b-FGF stimulates proliferation of chondrocyte, but the half-life is very short [11–13] and therefore, we used gelatin hydrogel sheets that incorporate b-FGF and allow gradual release for about 2 weeks as the sheets degrade [14]. Although several studies have shown that slow-release b-FGF stimulated reproduction of tracheal cartilage [16–18], to our knowledge, this is the first study which aimed to promote growth of the tracheal cartilage using this agent.

Traditionally, symptoms of children with moderate to mild tracheomalacia are thought to improve as they grow older [3–6] and is believed to be caused by a natural increase in the size of the tracheal lumen. Our current study showed that slow-release 5 μg of b-FGF increases the luminal area of the trachea and thickens the tracheal cartilage. These findings appear to be the result of chondrogenesis, and are consistent with other studies showing the role of b-FGF on chondrocyte proliferation [16–18]. Therefore, administration of slow-release b-FGF to the tracheal cartilage from the outside might make airway collapse difficult and become an alternative to invasive surgical interventions for patients with severe tracheomalacia. Tracheomalacia is often detected in patients with oesophageal atresia or those who have undergone slide tracheoplasty [19–22].

This study has several limitations. Firstly, this was a study performed in normal rats, not in rats with tracheomalacia. There are models for this disease, but larger animals such as piglets [23], sheep [24], and dogs [18] are used. In addition, such studies have used a surgical method of reproducing tracheomalacia by removing a portion of tracheal cartilage from consecutive rings. The ratio of cartilage to muscle is about 2–3 to 1 in patients with this disease, and that of normal children about 4:1 [22,25]. Our method, administration of slow-release b-FGF between the trachea and the esophagus, may cause elongation of both ends of the tracheal cartilage towards the membranous portion, and result in shortening of the widened membrane. The lack of a rat model for this disease limits further work. Secondly, although we showed rapid growth of the trachea with b-FGF, it remains unproven that the resultant trachea is more difficult to collapse. We are developing experimental settings to evaluate mechanical strength and collapsibility of the promoted tracheas. Finally, we still have to confirm the long-term effect of slow-release b-FGF. It may result in calcification of the tracheal cartilage or formation of a complete ring due to elongation of the trachea. The body weight of rats in the highest dose b-FGF (50 μg) group tended to be lower than those of the other groups and it may be that growth impairment was caused by tracheal stenosis.

Despite these limitations, our results showed the feasibility of growth promotion of the trachea using slow release b-FGF and suggested that there was an optimal dose for clinical use. Our current procedure, placement of the gelatin at the dorsal side of the membranous portion, is still invasive and far from clinical use. A minimally invasive administration method such as injection of b-FGF from the inside of trachea using bronchoscopy is under development.

Acknowledgment

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References


