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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 mm^2, 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.

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

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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, n=8 in all groups). General anesthesia was induced by inhalation of halothane and was maintained under spontaneous breathing without endotracheal intubation in the b-FGF groups (n=24). After a cervical midline incision, the cervical trachea was exposed and the area between the membranous portion of the trachea and the esophagus was dissected. Then, biodegradable gelatin hydrogel sheets that had incorporated 0.5, 5, or 50 μg/20 μl of b-FGF solution, respectively, were placed between the cervical trachea and the esophagus. Muscle and skin layers were separately closed using absorbable sutures. No intervention was performed in rats in the control group (n=8).

1.3. Pathological evaluation

Four weeks after the surgical procedure, all rats were sacrificed by intraperitoneal administration of a lethal dose of thiopental sodium, following inhalation anesthesia using halothane. The cervical tracheas were harvested and the specimens were embedded in Tissue-Tek OCT compound 4583 (Sakura Finetechnical Co. Ltd., Tokyo, Japan) and frozen. They were subsequently cut into 7-μm-sections and stained with hematoxylin and eosin (H & E), toluidine blue, and safranin O. The luminal area and the thickness of the cartilage were measured on cross-sections of each trachea using commercially available image processing software (Medical Image Analyzer, inotech Co. Ltd., Hiroshima, Japan). The thickness of the cartilage was defined as the value of the area of the cartilage divided by the length of its inner perimeter.

1.4. Statistical analysis

Statistical analysis was performed by Steel's method for nonparametric multiple comparison with control, using commercially available software (JMP™ 9.0.0, SAS Institute Japan Ltd., Tokyo, Japan). Data are expressed as mean (SD). P value of <0.05 was considered to be statistically significant.

2. Results

All rats in each groups survived until the time of sacrifice. However, due to a technical problem in cutting frozen specimens, we could not see the rings of the trachea completely in two of the eight specimens in both b-FGF 0.5 and 50 μg groups. These specimens were inappropriate for measurement, and were excluded from evaluations. Results of this study are shown in Table 1.

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.

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) mm^2, 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

Table 1 Results.

Group	Number of rats	Weight at the operation (g)	Weight at the sacrifice (g)	Luminal area (mm^2)	Thickness of the cartilage (mm)
Control	8	47 (12.6)	196 (22.5)	3.1 (0.3)	0.12 (0.01)
FGF 0.5	6	40 (2.6)	185 (12.3)	3.2 (0.3)	0.13 (0.01)
FGF 5	8	45 (5.2)	187 (8.8)	3.8 (0.6)*	0.19 (0.04)**
FGF 50	6	40 (2.3)	175 (7.2)	2.6 (1.3)	0.32 (0.08)**

Data are shown as mean (SD).
* $P<0.05$.
** $P<0.01$.

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.

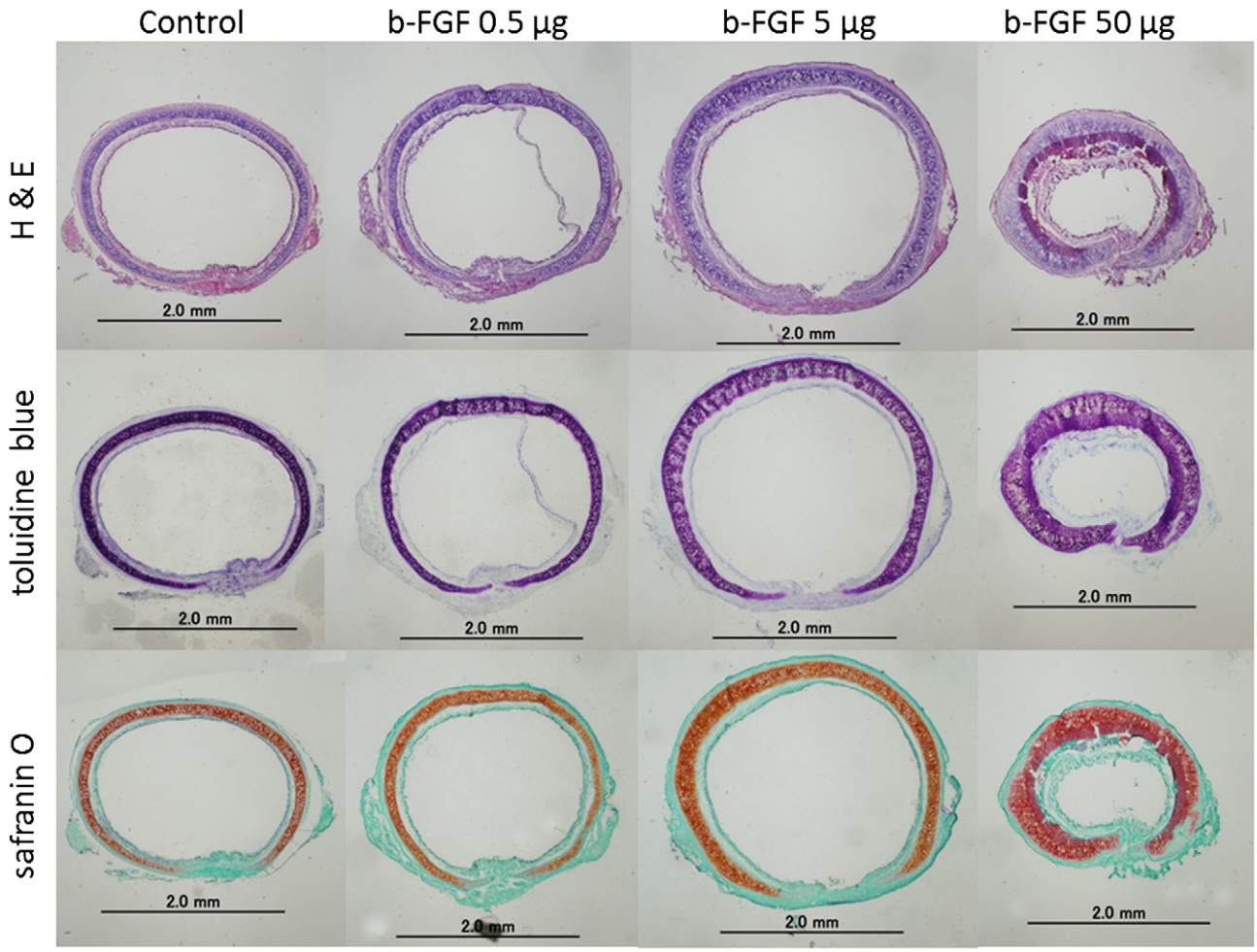

Fig. 1 Cross-section of the trachea in each group. The luminal area of the b-FGF 5 μg group is the largest and that of the b-FGF 50 μg group is the smallest of all groups. The thickness of the tracheal cartilage increases dose-dependently.

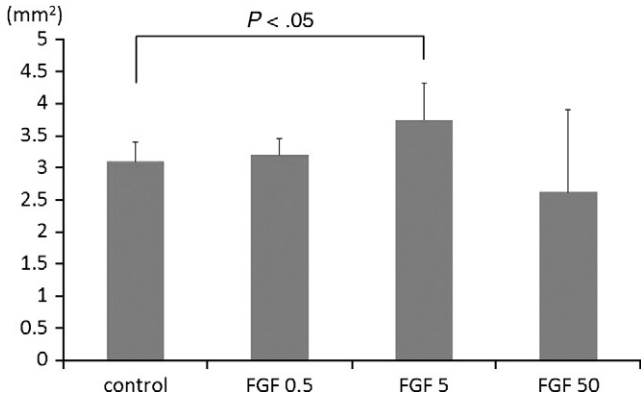

Fig. 2 Luminal area of the trachea. A bell-shaped dose–response curve with a peak at 5 μg of b-FGF is seen, and the luminal area of the FGF 50 μg group is smaller than that of the control group. A significant difference is seen only between the control group and the b-FGF 5 μg group.

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].

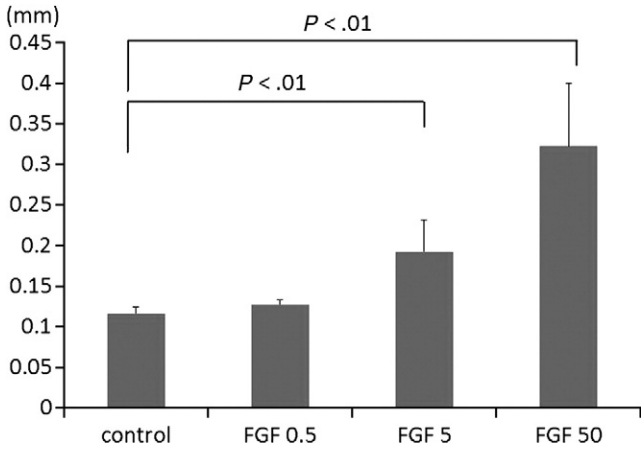

Fig. 3 Thickness of the tracheal cartilage. A dose-dependent increase is seen between the dose of b-FGF and the thickness of the tracheal cartilage.

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.

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References

[1] Masters IB, Chang AB. Interventions for primary (intrinsic) tracheomalacia in children. Cochrane Database Syst Rev 2005: CD005304.

[2] Pillai JB, Smith J, Hasan A, et al. Review of pediatric airway malacia and its management, with emphasis on stenting. Eur J Cardiothorac Surg 2005;27:35-44.
[3] Masters IB, Chang AB. Tracheobronchomalacia in children. Expert Rev Respir Med 2009;3:425-39.
[4] Doshi J, Krawiec ME. Clinical manifestations of airway malacia in young children. J Allergy Clin Immunol 2007;120:1276-8.
[5] Sommer D, Forte V. Advances in the management of major airway collapse: the use of airway stents. Otolaryngol Clin North Am 2000;33:163-77.
[6] Jacobs IN, Wetmore RF, Tom LW, et al. Tracheobronchomalacia in children. Arch Otolaryngol Head Neck Surg 1994;120:154-8.
[7] Przybylski M. A review of the current research on the role of bFGF and VEGF in angiogenesis. J Wound Care 2009;18:516-9.
[8] Nakajima H, Sakakibara Y, Tambara K, et al. Therapeutic angiogenesis by the controlled release of basic fibroblast growth factor for ischemic limb and heart injury: toward safety and minimal invasiveness. J Artif Organs 2004;7:58-61.
[9] Quatela VC, Sherris DA, Rosier RN. The human auricular chondrocyte. Responses to growth factors. Arch Otolaryngol Head Neck Surg 1993;119:32-7.
[10] Klagsbrun M, Smith S. Purification of a cartilage-derived growth factor. J Biol Chem 1980;255:10859-66.
[11] Ellman MB, An HS, Muddasani P, et al. Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene 2008;420:82-9.
[12] Lazarous DF, Shou M, Stiber JA, et al. Pharmacodynamics of basic fibroblast growth factor: route of administration determines myocardial and systemic distribution. Cardiovasc Res 1997;36: 78-85.
[13] Edelman ER, Nugent MA, Karnovsky MJ. Perivascular and intravenous administration of basic fibroblast growth factor: vascular and solid organ deposition. Proc Natl Acad Sci U S A 1993;90: 1513-7.
[14] Isogai N, Morotomi T, Hayakawa S, et al. Combined chondrocyte-copolymer implantation with slow release of basic fibroblast growth factor for tissue engineering an auricular cartilage construct. J Biomed Mater Res A 2005;74:408-18.
[15] Tabata Y, Nagano A, Ikada Y. Biodegradation of hydrogel carrier incorporating fibroblast growth factor. Tissue Eng 1999;5:127-38.
[16] Tatekawa Y, Kawazoe N, Chen G, et al. Tracheal defect repair using a PLGA-collagen hybrid scaffold reinforced by a copolymer stent with bFGF-impregnated gelatin hydrogel. Pediatr Surg Int 2010;26:575-80.
[17] Komura M, Komura H, Kanamori Y, et al. An animal model study for tissue-engineered trachea fabricated from a biodegradable scaffold using chondrocytes to augment repair of tracheal stenosis. J Pediatr Surg 2008;43:2141-6.
[18] Igai H, Yamamoto Y, Chang SS, et al. Tracheal cartilage regeneration by slow release of basic fibroblast growth factor from a gelatin sponge. J Thorac Cardiovasc Surg 2007;134:170-5.
[19] Castilloux J, Noble AJ, Faure C. Risk factors for short- and long-term morbidity in children with esophageal atresia. J Pediatr 2010;156: 755-60.
[20] Carden KA, Boiselle PM, Waltz DA, et al. Tracheomalacia and tracheobronchomalacia in children and adults: an in-depth review. Chest 2005;127:984-1005.
[21] Tsugawa C, Nishijima E, Muraji T, et al. Tracheoplasty for long segment congenital tracheal stenosis: analysis of 29 patients over two decades. J Pediatr Surg 2003;38:1703-6.
[22] Wailoo MP, Emery JL. The trachea in children with tracheo-oesophageal fistula. Histopathology 1979;3:329-38.
[23] Vinograd I, Filler RM, England SJ, et al. Tracheomalacia: an experimental animal model for a new surgical approach. J Surg Res 1987;42:597-604.
[24] Tsukada H, O'Donnell CR, Garland R, et al. A novel animal model for hyperdynamic airway collapse. Chest 2010;138:1322-6.
[25] Mair EA, Parsons DS. Pediatric tracheobronchomalacia and major airway collapse. Ann Otol Rhinol Laryngol 1992;101:300-9.