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Therapeutic Efficacy of Basic Fibroblast Growth Factor in Patients With Vocal Fold Atrophy

Ayako Okui, MD ©; Ujimoto Konomi, MD, PhD; Takeharu Kanazawa, MD, PhD ©; Daigo Komazawa, MD; Kazuhiro Nakamura, MD, PhD; Koji Matsushima, MD, PhD ©; Yusuke Watanabe, MD, PhD

**Objectives/Hypothesis:** In recent years, basic fibroblast growth factor (bFGF) injection has been used in the treatment of aging-related vocal fold atrophy. This injection not only improves closure by increasing the mass of the vocal fold but also improves its viscoelasticity. However, it has been reported that fibroblasts targeted by bFGF treatment decrease in number with age. The purpose of this study was to examine the effects of local injection of bFGF on age-related vocal atrophy as well as the influence of age on phonological outcomes.

**Study Design:** Retrospective chart review.

**Methods:** Fifty-three patients with age-related vocal fold atrophy underwent single injections of bFGF in their vocal folds. Phonological outcomes were evaluated 3 and 6 months after injection by acoustic and aerodynamic measurements. From the Tokyo Voice Center (A.O., U.K., T.K., D.K., K.N., K.M., Y.W.), International University of Health and Welfare Graduate School, Tokyo; Department of Otolaryngology–Head and Neck Surgery (K.N.), Nihon University, Itabashi Hospital, Tokyo; and the Department of Otolaryngology–Head and Neck Surgery (K.m.), Toho University, Omori Medical Center, Tokyo, Japan. Editor’s Note: This Manuscript was accepted for publication on January 13, 2020.

Age-related vocal atrophy is a pathological condition mainly characterized by the thinning of the lamina propria and atrophy of the vocal fold muscles. In the lamina propria, the superficial layer becomes thinner due to decreased hyaluronic acid, and fibrosis due to accumulation of denatured collagen causes dysphonia due to abnormal viscoelasticity of the vocal folds. In addition, it has been reported that a reduction in the vocal fold muscle cross-sectional area starts at 50 years of age and decreases significantly after 80. A spindle-shaped gap at the time of vocalization due to muscle atrophy and thinning of the lamina propria increases breathiness, giving the impression of a voice of a more aged person.

For hoarseness due to vocal fold atrophy, surgical treatments such as local injection of collagen or hyaluronic acid into the vocal fold, intravocal injection of autologous fat, and type I thyroplasty have been performed. There are also reports of voice therapy for vocal fold atrophy, but with limited success.

In recent years, research on regenerative medicine has advanced the field of phonosurgery. Hirano et al. reported that basic fibroblast growth factor (bFGF) promotes the production of hyaluronic acid in the vocal folds in vitro and in vivo. In 2008, bFGF treatment for vocal fold atrophy in humans due to aging was first reported. Since then, several follow-up tests have been conducted on the local injection of bFGF for vocal fold atrophy, and good results have been obtained albeit in a small population. In this study, we compared not only the effect of treatment but also the effect on elderly individuals.

**INTRODUCTION**

Language is a very important tool in communication, and phonatory function plays a very important role. However, phonatory function declines with age, and communication disorders caused by dysphonia are important issues that can decrease quality of life in elderly people and can lead to isolation. According to the 2017 World Population Prospects by the United Nations, 17.6% of the global population was aged 65 years and over in developed areas as of 2015, and that number is expected to reach 27.6% by 2060. Roy et al. reported that the prevalence of dysphonia associated with people 65 years or older was 29.3%. Thus, now that the elderly population is increasing worldwide, dysphonia in elderly individuals, including age-related vocal fold atrophy, is a very common disease.

**Key Words:** Basic fibroblast growth factor, vocal fold atrophy, elderly patients, regenerative medicine.

**Level of Evidence:** 2c

**Laryngoscope, 130:2847–2852, 2020**
MATERIALS AND METHODS

Patients
The subjects were patients who presented to the Tokyo Voice Center over 3 years starting in April 2014 who had hoarseness caused by age-related vocal fold atrophy. Patients without age-related atrophy (e.g., atrophy after inhaled steroids, atrophy due to paralysis) were excluded. Patients over the age of 50 years without head and neck cancer and without serious complications were included in the treatment. Patients who participated in this treatment received adequate informed consent and agreed to the treatment. This study was conducted in accordance with the Helsinki Declaration and was approved by the ethics committee (institutional review board number: 14-S-3).

Drug Information
A commercially available human recombinant bFGF preparation (Fiblast; Kaken Pharmaceutical, Tokyo, Japan) was used. This drug was approved by the Ministry of Health, Labor and Welfare (Tokyo, Japan) in 1991 for the treatment of pressure ulcers and skin ulcers in the dermatological field. It is used as a spray for skin lesions, and so far, no serious side effects have been reported. The lyophilized product was supplied with the included proprietary solution, and it was dissolved and used with the included solution in this treatment.

Injection Protocol
Injection of bFGF was performed under local anesthesia. After oral inhalation of 4% lidocaine, the surfaces of the pharyngeal and laryngeal mucosa were anesthetized with 4% lidocaine. While observing the larynx with a nasal fiberscope, bFGF was injected into the vocal folds with a 23-gauge injection needle (Varixer; TOP, Tokyo, Japan). A solution of 100 μg bFGF dissolved in 1.0 mL of the solution was injected into the lamina propria at 0.5 mL per side. The concentration of the drug was equivalent to the concentration used for skin ulcers, and the amount used was less than 10% of the manufacturer's indicated maximum daily dose. The patient was instructed not to speak for 24 hours after the operation and was allowed to speak starting from the next day. We observed patients in the hospital for 1 hour after injection and confirmed that there were no allergic reactions or serious side effects.

Assessment
Evaluations of vocal outcomes were performed 3 and 6 months after injection. Maximum phonation time (MPT), mean flow rate (MFR), pitch range, noise-to-harmonic ratio (NHR), jitter, shimmer, and Voice Handicap Index (VHI) were evaluated. MFR was measured with a PS-77E phonatory function analyzer (Nagashima Medical Instruments, Tokyo, Japan). Acoustic analysis (jitter, shimmer, and NHR) was evaluated by a computerized speech lab model 4500 (KayPENTAX, Montvale, NJ). Statistical analysis of the VHI and each parameter from aerodynamic examinations and acoustics analyses before and after treatment was performed by one-way repeated measures analysis of variance with a post hoc Scheffe test. A P value < .05 was considered statistically significant. Statistical analysis was performed using SPSS (version 25; IBM, Armonk, NY).

RESULTS
A total of 53 patients (41 males, 12 females) were enrolled, and bFGF was injected into their vocal folds. The mean age was 69.0 ± 9.5 years (male 68.0 ± 9.9, female 74.5 ± 8.3 years). Twenty-four patients were older than 70 years and 29 were younger than 70 years. No cases of allergic reaction after bFGF injection were noted, and no serious side effects were observed within the observation period.

Voice Handicap Index
Figure 1A shows the mean VHI and standard deviation at 3 and 6 months after surgery, respectively. The VHI showed a marked decrease at 3 months after surgery (P < .001) and maintained a significantly low level at 6 months after surgery (P < .001).

Aerodynamic Examination
Figure 1B and C show the change of the MPT and MFR before and after surgery. The MPT showed improvement after surgery (P < .001), and the effect continued.
6 months after surgery ($P < .001$). The MFR showed a mean decrease, but this was not significant ($P = .246$).

**Acoustic Analysis**

Figure 2 indicates sequential changes in jitter, shimmer, and NHR before and after surgery. Jitter and shimmer showed improvement at 3 months after surgery ($P < .001$ and $P = .006$, respectively), and the effect continued at 6 months after surgery ($P < .001$ and $P = .02$, respectively). However, NHR did not show a significant improvement after surgery ($P = .246$).

**Pitch range**

Compared with preoperative levels, the postoperative pitch range was greatly improved ($P < .001$) (Fig. 3). The lower end of the range fell from 114.5 to 102.4 Hz, the upper end of the range increased from 452 to 510.7 Hz, and the pitch range expanded. The speaking fundamental frequency dropped from 172.6 to 159.3 Hz.

**Therapeutic effect in individuals over 70 years old**

Figure 4 shows the change of the VHI and MPT before and after surgery in those over 70 years old and those under 70 years old. Even in those over 70 years old, VHI shows a marked improvement at 3 and 6 months after surgery ($P < .001$ and $P < .001$, respectively). In addition, the degree of improvement in VHI was not significantly different between age groups ($P = .215$). Similarly, MPT in those over 70 years old showed improvement at both 3 and 6 months after surgery ($P < .001$ and $P < .001$, respectively).

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**Fig. 2.** Acoustic parameters at preinjection and 3 and 6 months postinjection. (A) Jitter. (B) Shimmer. (C) Noise-to-harmonic ratio (NHR). $^{*}P < .001$, $^{**}P = .006$, $^{***}P = .02$; one-way repeated measures analysis of variance with post hoc Scheffe test.

**Fig. 3.** (A) Pitch range at reinjection and 3 and 6 months postinjection. $^{*}P < .001$, one-way repeated measures analysis of variance with post hoc Scheffe test. (B) Preinjection and 3 months postinjection values of the upper and lower end of the range, speaking fundamental frequency. $^{*}P < .001$, $^{**}P = .002$; paired t test.
The improvement rate of MPT in those over 70 years old was 47.06% and under 70 years old was 47.37%. There was no significant difference in this improvement rate between the groups ($P = .983$).

**DISCUSSION**

Various treatments such as voice therapy, hyaluronic acid injection, collagen injection, autologous fat injection, and type I thyroplasty have been performed for age-related vocal fold atrophy. In treating vocal folds through injection, fillings with viscoelasticity that are readily available, not cumbersome, highly stable, and do not interfere with vocal fold vibrations are ideal, but no material meets these criteria. Hyaluronic acid and collagen are easily obtained because they are supplied as medical materials, and the procedure is also simple. Hyaluronidase injection in vocal folds can correct overinjection of hyaluronic acid. However, because hyaluronidase is absorbed, it has a short activity duration and requires multiple treatments. Collagen is a heterogeneous protein, and allergic reactions to it cannot be ignored. Fat is a self-organizing, nonirritating, and safe filling, but it does not effectively improve vibration, and it has been reported that the effect diminishes within 1 year after the second half of the operation. Type 1 thyroplasty requires a percutaneous incision and is more invasive than vocal fold injection. The injection of bFGF was simple and, as shown in Table I, satisfactory improvement was observed even when compared with other injections.

The treatment described above is for mass correction of the vibrating part of the vocal folds and the inward movement of vocal folds for glottal insufficiency. However, bFGF injection into the vocal folds not only increases vocal fold mass, but also regenerates the layer structure of the vocal fold lamina propria mucosa and restores its viscoelasticity. This is an antiaging regenerative approach for the voice and is a more radical treatment. In addition, it has been proven that bFGF injection not only increases hyaluronic acid in the lamina propria and improves mucosal wave motion, but also increases the mass of the thyroarytenoid muscle. Kaneko et al. reported that the muscle tissue mass increased due to bFGF injection into the thyroarytenoid muscle after recurrent laryngeal nerve transection. This is because bFGF acts as a signaling factor in the

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Fig. 4. Voice Handicap Index (VHI) and maximum phonation time (MPT) of preinjection and 3 and 6 months postinjection in those over 70 years old and under 70 years old. *$P < .001$, one-way repeated measures analysis of variance with post hoc Scheffe test.
activation of satellite cells of myocytes and their differentiation into myoblasts. In this study, the injection site targeted the lamina propria of the vocal fold. At the time of injection, we punctured the vocal folds slightly deeper and injected while pulling the needle out. Based on the observation that the vocal folds swelled like a balloon, it was determined that the injection into the lamina propria was successful. Therefore, some bFGF was injected into the thyroarytenoid muscle during the injection process, and it was thought that the effect of increasing the thyroarytenoid muscle improved glottal closure.

For the first time in 2008, Hirano et al. performed bFGF vocal fold injection on age-related vocal atrophy and confirmed its effect. Since then, several studies have been conducted. This effect has been confirmed, and no adverse events were observed in any reports. In our study, postoperative improvement was observed in all items except NHR and MFR.

Although the parameters of improvement differ slightly, all studies saw improvements. In addition, Hirano et al. and Suzuki et al. reported long-term results over the course of 1 year after treatment with bFGF. Hirano et al. reported a sustained treatment effect on pitch perturbation quotient, amplitude perturbation quotient, NHR, and MPT 1 year after surgery. Suzuki et al. confirmed that the grade and roughness of the GRBAS (Grade, Roughness, Breathiness, Asthenia, and Strain) score and MPT continued to be effective even after 1 year. These are all good results, but we think that it is necessary to follow up with patients for 1 year or more after the operation.

In this study, in addition to evaluating pre- and postoperative treatment effects, we compared the effects in patients over 70 years old and those under 70 years old. Both groups showed significant improvement after surgery, but the improvement rate of MPT was not significantly different between groups.

Sato and Hirano reported that fibroblasts decreased in the vocal folds of elderly patients. If fibroblasts stimulated by bFGF and producing hyaluronic acid decreased in elderly patients, the treatment improvement rate was also expected to decrease; however, there was no significant difference. There was also no significant difference between the two groups in VHI, and it was determined that this surgery is effective for elderly patients.

In our study, bFGF was injected into the lamina propria mucosa in the middle of the vocal folds. However, there were reports that the fibroblasts of the vocal fold macular flava more actively produce extracellular matrix than fibroblasts in Reinke’s space, and so it is necessary to consider the selection of the injection site.

There were also cases in which sufficient effects were not obtained. We used a single injection in this study. Because bFGF is a stimulating factor of fibroblasts, we would like to investigate whether the effect can be obtained by multiple injection and repeat the stimulation in cases where the effect is not obtained. In addition, there is a report that thyroarytenoid muscle atrophy in rats can be improved by a high-dose injection of bFGF, and so investigations into dose-dependent effects are warranted.

The therapeutic effect of a single injection of bFGF for vocal fold atrophy in our study showed subjective and objective improvements similar to previously reported results with multiple injections. Many patients with age-related vocal fold atrophy are elderly, and it is not uncommon for them to have multiple underlying diseases. In this study, it was found that even a single injection can provide a sufficient effect, and this treatment can be performed with local anesthesia, which is thought to reduce the burden on elderly patients.

CONCLUSION

The number of elderly people with age-related vocal fold atrophy is increasing with the aging population. A single injection of bFGF to the vocal folds improved both objective and subjective findings. Most patients with vocal fold atrophy are elderly, and it is not uncommon for them to have many underlying diseases. This method of treatment can be performed with local anesthesia that is not easily affected by underlying disease and is minimally invasive. Previous studies have not evaluated the effect of this treatment according to age. In this study, this treatment method obtained sufficient therapeutic effects even in elderly people. We hope that this treatment will become widely adopted in practices around the world.

BIBLIOGRAPHY


