Protective Effect of Astaxanthin on Vocal Fold Injury and Inflammation Due to Vocal Loading: A Clinical Trial

*Mami Kaneko, *Yo Kishimoto, *Ryo Suzuki, *Yoshitaka Kawai, *Ichiro Tateya, and †Shigeru Hirano, *†*Kyoto, Japan*

Summary: Objectives. Professional voice users, such as singers and teachers, are at greater risk of developing vocal fold injury from excessive use of voice; thus, protection of the vocal fold is essential. One of the most important factors that aggravates injury is the production of reactive oxygen species at the wound site. The purpose of the current study was to assess the effect of astaxanthin, a strong antioxidant, on the protection of the vocal fold from injury and inflammation due to vocal loading.

Study Design. This study is an institutional review board-approved human clinical trial.

Methods. Ten male subjects underwent a 60-minute vocal loading session and received vocal assessments prior to, immediately after, and 30 minutes postvocal loading (AST(−) status). All subjects were then prescribed 24 mg/day of astaxanthin for 28 days, after which they received the same vocal task and assessments (AST(+) status). Phonatory parameters were compared between both groups.

Results. Aerodynamic assessment, acoustic analysis, and GRBAS scale (grade, roughness, breathiness, asthenia, and strain) were significantly worse in the AST(−) status immediately after vocal loading, but improved by 30 minutes after loading. In contrast, none of the phonatory parameters in the AST(+) status were statistically worse, even when measured immediately after vocal loading. No allergic responses or adverse effects were observed after administration of astaxanthin.

Conclusions. The current results suggest that astaxanthin can protect the vocal fold from injury and inflammation caused by vocal loading possibly through the regulation of oxidative stress.

Key Words: Astaxanthin–Vocal fold–Vocal loading–Reactive oxygen species–Clinical trial.

INTRODUCTION

Excessive use or abuse of the voice can lead to symptoms of hoarseness, weak voice, sore throat, and dysphonia. It has been suggested that professional voice users, such as teachers, singers, sales professionals, or Buddhist priests, are more at risk of developing vocal disorders than others.[1] In general, conservative treatment methods such as voice therapy—mainly consisting of voice rest, vocal hygiene, and medical management—are used as the first line of treatment. However, voice rest is often difficult in these occupations and adequate voice therapy cannot be performed, making treatment difficult.[2] Thus, the development of more useful and scientifically validated treatments is greatly needed.

Kojima et al[3] quantified the extent of epithelial surface damage within the vocal fold following vibration exposure using an *in vivo* rabbit phonation model. They applied varying phonation times (30, 60, or 120 minutes) and magnitudes (control, modal intensity phonation, or raised intensity phonation) of the vibration exposure. Scanning and transmission electron microscopic examinations revealed a significant reduction in microprojection density, microprojection height, and depth of the epithelial surface, with increasing time and phonation magnitude. These results suggest an increased risk of epithelial surface damage with excessive and prolonged vibration exposure. Swanson[4] also reported that a transient episode of raised-intensity phonation caused a significant increase in the expression of vocal fold inflammatory messenger RNA (mRNA), including interleukin (IL)-1β, COX-2, and TGFβ1, compared with modal phonation and sham control in an *in vivo* rabbit phonation model.

Reactive oxygen species (ROS) are normal byproducts of cellular metabolic processes, including the mitochondrial respiratory chain.[5] Although ROS is essential for various signaling pathways,[6] overexposure to ROS, referred to as oxidative stress, is a harmful process that can be a significant source of damage to cellular components, including lipids, proteins, and DNA. ROS overexposure can also contribute to cellular aging and malignant transformation.[7]

A large amount of ROS is produced by inflammatory cells via nicotinamide adenine dinucleotide phosphate oxidase, which plays a central role in the defense against wound infection.[8] However, prolonged oxidative and inflammatory stress following skin wounding is thought to be associated with excessive scarring.[9]

Mizuta et al[10] suggested that a large amount of ROS is produced during the early phase of vocal fold wound healing, until postinjury day 3, and this period may be crucial for regulating ROS levels. They also suggested that inflammatory cells may contribute to ROS generation. Mizuta et al[11] also investigated the effect of astaxanthin on the regulation of oxidative stress and scarring during vocal fold wound healing. Their results suggested that astaxanthin has the potential to prevent vocal fold scarring by regulating oxidative stress during the early phase of healing.

Astaxanthin is a xanthophyll carotenoid found in various microorganisms and marine animals.[7,12–14] Astaxanthin has a strong antioxidant effect as a potent quencher of singlet oxygen, and many studies have shown favorable effects of astaxanthin on the regulation of oxidative stress.[7,13,15–20] Several studies have also

Accepted for publication June 28, 2016.
Financial disclosure: None.
Conflict of interest: None.
From the *Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; and the †Department of Otolaryngology Head & Neck Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan.
Address correspondence and reprint requests to Shigeru Hirano, Department of Otolaryngology Head & Neck Surgery, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan. E-mail: hirano@koto.kpu-m.ac.jp
Journal of Voice, Vol. 31, No. 3, pp. 352–358
0892-1997

http://dx.doi.org/10.1016/j.jvoice.2016.06.017

reported on the administration of astaxanthin for human health purposes and have confirmed its antioxidant activity and safety.[21,22] However, there are no clinical studies that assessed the effect of astaxanthin on vocal fold injury or inflammation. The purpose of the current clinical study was to assess the effects of astaxanthin on the protection of the vocal fold from injury and inflammation due to vocal loading.

METHODS

Subjects

Ten non-singer male subjects participated in the study: the age range was 23–30 years old and the mean age was 24.2 years. All subjects were in good overall health based on self-reports and had no history of major systemic diseases or laryngeal surgery. Any vocal fold lesions or functional problems were excluded by laryngoscopic examination. In addition, participants who used any medications that might influence voice (eg, diuretics, decongestants, and antihistamines)[23] or with a history of a smoking habit were excluded.

Procedures

The general experimental paradigm is shown in Figure 1.

Day 0

All subjects first received vocal assessments, as described in detail below. Subjects then underwent a 60-minute vocal loading session with reading a Japanese novel. During loading, subjects alternated between 15 minutes of loud phonation with 5 minutes of rest, for a total of three cycles over the 60-minute loading period.[24] Immediately after the 60-minute vocal loading session, subjects received the same vocal assessments as performed prior to loading. Subjects then rested for 30 minutes. Subjects were allowed to drink water during the break period after the vocal load task and after completion of loading, but were not allowed to talk during the break. After 30 minutes of rest, subjects received the vocal assessments again. After all vocal assessments and vocal loading were completed, subjects received guidance to take 24 mg/day of astaxanthin for 28 days and to refrain from vocal abuse for 1 month. We referred to the data obtained prior to taking astaxanthin as the "AST(−) status".

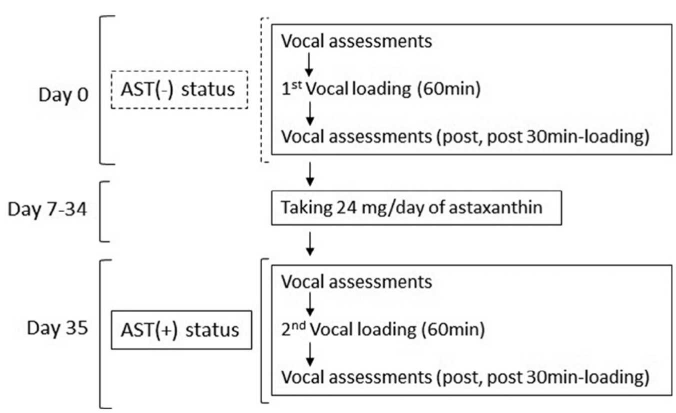

FIGURE 1. General experimental paradigm.

Days 7–34

All subjects took 24 mg/day of astaxanthin. We confirmed the condition of their health and compliance with taking astaxanthin via phone interviews every week after they started treatment.

Day 35

All subjects received the same vocal assessments and vocal loading that were performed on Day 0. Data obtained after the subjects took astaxanthin are referred to as the "AST(+) status". During vocal loading, a sound level meter (NL-42; RION Co., Tokyo, Japan) was positioned at a constant distance of 25 cm from the subject's mouth so that the experimenter could monitor relative intensity levels during loading. The examiner monitored the meter nearly constantly and cued subjects to maintain a target intensity range of 75–90 dB during phonatory loading.[24] All procedures were approved by the institutional review boards of Kyoto University.

Assessment

Vocal outcomes were evaluated prior to, immediately after, and 30 minutes postvocal loading. The following assessments were performed: GRBAS scale (grade, roughness, breathiness, asthenia, and strain), aerodynamic assessment, acoustic analysis, intensity, pitch range, stroboscopic examinations, and the Vocal Fatigue Index (VFI). Stroboscopic examination was performed using a digital video stroboscopy system with a 70° rigid endoscope, Model 9295 (KayPENTAX, Lincoln Park, NJ). Two raters blinded to AST(+) and AST(−) status assessed all the stroboscopic images of the larynx at the same time.

Aerodynamic assessments, which included maximum phonation time (MPT), mean flow rate (MFR), phonation threshold pressure (PTP), intensity, and pitch range, were examined with a phonation analyzer at the patient's normal pitch and loudness (PA-500; Nagashima Co., Osaka, Japan). Acoustic analyses evaluated jitter and shimmer using the Multidimensional Voice Program (Model 5105, KayPENTAX).

The GRBAS scale is an anchored perceptual analysis. GRBAS was independently evaluated by two trained raters, a laryngologist and a speech language pathologist. This scale was first developed by the Japanese Society of Logopedics and Phoniatrics and has become popular worldwide.[25] The GRBAS scale is scored from 0 to 3 in which 0 = within normal limits, 1 = slight, 2 = moderate, and 3 = severe. The ratings on the five subscales (G, R, B, A, and S) were summed, and two raters blinded to AST(+) and AST(−) status assessed all GRBAS scales at the same time. The mean rating score between the two raters was calculated. Inter-rater reliability was evaluated using Spearman's rank correlation coefficient, and the results showed significant correlation between the two, with a correlation coefficient of $r = 0.8$ ($P < 0.001$).

The VFI is a standardized tool that can identify individuals with probable vocal fatigue with good reliability, validity, sensitivity, and specificity.[26]

Statistical test

Statistical analysis was performed using commercially available software (*Excel Statistics 2012*; Social Survey Research