The Effect of Aging on Blood Flow in Rat Larynx

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Abstract

1. Introduction

Aging affects vocal function[1-3] These changes in function may be related to musculoskeletal changes, such as alterations in neuromuscular structure or contractile properties,[4, 5] or may be due to changes in the viscoelasticity of vocal fold mucosa extracellular matrix.[6-9] However, little is known concerning the potential mechanisms of age-related vocal fold pathophysiology.

A contributing factor to age-related changes in vocal function may be a compromise in blood flow to laryngeal mucosa and muscle. In contrast to spinal motor systems, there is a dearth of knowledge concerning the effects of aging on laryngeal blood flow. With aging, alterations in nutritive blood flow and oxygen supply may be associated with changes in phonatory function. Reductions in blood flow to laryngeal muscle could translate to reductions in oxidative capacity within laryngeal muscles, reductions in endurance capabilities, and increased fatigability. Reduced perfusion of lamina propria may contribute to altered tissue viscosity and resultant vibratory properties. Quantifying changes in the vascular blood supply to laryngeal tissue will further the understanding of age-related decline in laryngeal sensorimotor function.

Until recently, questions concerning microcirculatory hemodynamics in the larynx were technically difficult to answer. The purpose of our study was to develop a new technique for *in vivo* imaging of blood flow using an intravital microscopy technique, and to investigate possible differences in microcirculation as a function of aging. Our hypothesis was that age-associated changes in laryngeal blood flow would be manifested as reductions in perfusion of laryngeal tissue.

2. Methods

To develop the *in vivo* laryngeal blood flow imaging technique, intravital microscopy was used to visualize and record critical aspects of laryngeal microcirculation in 10 male Fisher 344/Brown Norway rats. The rats were equally distributed between two age groups: young adult (9 mo. old) and old (28-30 mo. old).

The larynx was surgically exposed to allow *in vivo* recording of laryngeal blood flow, A laryngofissure was created by carefully cutting the cricoid and thyroid cartilages at the midline without damage to the intrinsic laryngeal muscles and overlying mucosa, and pulling the medial edges of the vocal folds laterally. We then attached sutures to the lateral margins at the cricoid cartilage and the top of thyroid cartilage, and pulled the sutures laterally to obtain an optimal laryngeal position that was nearly flat for use in intravital microscopy.

Blood plasma was labeled with a 5% fluorescein isothiocyanate (FITC)-labeled dextran 70 via a cannula in the left femoral artery. Fluorescence was excited with a xenon arc lamp (excitation wavelength 420 to 490 nm; emission wavelength, 520 nm) for visualization of the vascular bed. Using a microscope specially equipped to capture images in a live animal, measurements of left vocal fold capillary geometry and hemodynamics were obtained, including the speed (μm/sec) of red blood cells (RBCs) as they moved through small blood vessels; capillary density; tortuosity as a measure of capillary surface area, and percentage of flowing capillaries.

3. Results

We developed an animal for examining laryngeal blood flow in living animals. A representative still image is shown in Figure 1. A video clip can be viewed at http://www.surgery.wisc.edu/Oto/faculty/connor.shtml, under Research Interests, ICVPB 2004. In the video, laryngeal blood flow is viewed at 15 frames/sec and specifications of microscope images are same as those described in Figure 1. Results specific to age-related differences in microcirculatory hemodynamics will be presented at the meeting.

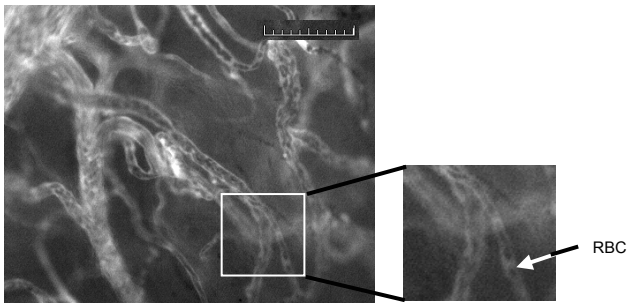

Figure 1: Microscopic images of young rat larynx microvasculature obtained via fluorescence intravital microscopy with a 20X objective and a X2 magnification changer yielding a 415 (W) X 335 (H) μm field. The region of laryngeal tissue imaged is left rat vocal fold, including lamina propria and underlying medial thyroarytenoid muscle. The left frame shows a single field using fluorescein isothiocyanate-dextran (FITC-dextran 150) and was detected with fluorescence intensity (excitation wavelength 420 to 490 nm; emission wavelength, 520 nm)**. The smaller right frame contains a d**igitally enhanced detail for visualization of an individual capillary and red blood cell at the arrowhead. Scale bar = 100 μm.

4. Discussion

We have developed a new technique for imaging blood flow to the larynx in an *in vivo* model. We will use this model for investigating age-related changes in vascular patterns and dynamics. The model we have developed may provide more information on laryngeal hemodynamics than standard electron microscopy in that we are able to visualize the geometry of microvasculature within larynx *in vivo* . In addition, the intravital method allows visualization of actual blood flow patterns within the microvasculature.

As we further develop our *in vivo* imaging model, we hope to provide further understanding of alterations in laryngeal capillary hemodynamics that occur in aging. The results of this preliminary study may contribute to development of further hypotheses concerning mechanisms of vocal function changes aging larynx, and to testing of potential treatments.

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6. References

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