Characterization of Submucosal Lesions Using Optical Coherence Tomography in the Rabbit Subglottis

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Objective: To evaluate the efficacy of optical coherence tomography in differentiating between several simulated subglottic lesions, using an ex vivo rabbit laryngotracheal model.

Design: Laryngotracheal complexes were harvested from euthanized rabbits and divided into the following 4 groups: (1) control, (2) submucosal collagen injection (simulating scar formation), (3) dehydration and rehydration (simulating edema), and (4) repeated intubation trauma. The subglottic region was imaged using optical coherence tomography. Images were later correlated with conventional histologic findings.

Results: The epithelium, basement membrane, lamina propria, perichondrium, and cartilage (cricoid and tracheal) were clearly imaged. In group 2, an increase in the thickness of the lamina propria was observed, in addition to a characteristic optical pattern of the injected collagen. Dehydration (in group 3) produced a visible reduction in the thickness of the lamina propria, while rehydration of the same specimen with distilled water revealed a significant increase in submucosal swelling. Repeated intubation (in group 4) resulted in tissue edema that was seen as wavy heterogeneous thickening of the lamina propria. Edema produced by repeated intubation or distilled water immersion was easily differentiated from native and collagen-injected tissues.

Conclusion: Optical coherence tomography successfully identifies the microstructure layers of the subglottis and can differentiate between edema and increased collagen deposition in the rabbit model.


ADVANCES IN MEDICAL TECHNOLOGY IN NEONATAL INTENSIVE CARE HAVE RESULTED IN THE EXTENSIVE USE OF PROLONGED ENDOTRACHEAL INTUBATION FOR VENTILATORY SUPPORT.1 This trend has led to a substantial increase in the incidence of acquired subglottic stenosis affecting the premature neonate.1-7 The diagnosis of subglottic stenosis is generally made during surgical endoscopy that is performed to evaluate the airway after failed attempts at extubation or in the presence of persistent stridor following extubation. Endoscopy remains the gold standard modality used to evaluate suspected intraluminal pathologic conditions. Unfortunately, differentiating between edema, scar tissue, or neocartilage development a priori with endoscopic evaluation is imprecise. Furthermore, endoscopic assessment of the infant airway is a challenging and potentially threatening procedure because of underlying lung disease, increased airway sensitivity to stimuli, vulnerability to additional airway trauma with associated edema, and the higher metabolic rate, requiring shorter periods of hypoxia during evaluation.

Despite their noninvasive benefits, other current imaging technologies, including computed tomography, magnetic resonance imaging, and ultrasonography, lack the necessary spatial resolution and ability to distinguish between scar tissue and edema in the subglottis of small subjects. The development of an imaging modality that can diagnose the histopathologic cause of subglottic stenosis in an indirect manner, without the risks associated with surgical endoscopy, could significantly affect management of these patients.

Optical coherence tomography (OCT) is an emerging imaging technology that combines light from a low-coherence source with a Michelson interferometer to produce cross-sectional images of tissue structures, with resolution approaching
that of light microscopy, typically 10 to 15 µm. Optical coherence tomography systems have been used clinically for ophthalmologic, intravascular, and skin imaging. However, there is limited information about the application of this modality in the assessment of the upper aerodigestive tract and no discussion of subglottic pathologic conditions. In this pilot study, we evaluated the usefulness of OCT in differentiating between various simulated submucosal lesions in the ex vivo rabbit subglottis.

METHODS

SPECIMENS

Laryngotraheal complexes were harvested from 7 New Zealand White rabbits that were euthanized for other institutional animal care and use committee-approved protocols at the University of California, Irvine. The specimens were dissected in the anteroposterior dimension (Figure 1) and then placed in isotonic sodium chloride solution. The specimens were divided into the following 4 groups: (1) control, (2) submucosal collagen injection (simulating scar formation), (3) dehydration and rehydration (simulating edema), and (4) intubation trauma. Figure 2 is a flow diagram outlining the experimental steps. In group 2, submucosal scar tissue was simulated by injecting collagen (Zyderm I; Inamed, Santa Barbara, Calif) into the submucosal region of the rabbit subglottis with a 30-gauge needle. The specimens were immediately imaged using OCT. In group 3, dehydration of specimens was produced with the immersion of the laryngotraheal complex in a 5% sodium chloride solution for 25 minutes. The specimens were immediately imaged using OCT after removal from the isotonic sodium chloride bath to establish a baseline state of maximal tissue dehydration. Following the initial imaging, a
A hypotonic solution (distilled water) was then placed over the specimen, while real-time OCT images were taken every 5 minutes for 25 minutes. The final image taken after 25 minutes of rehydration represents a state of maximum tissue edema. The specimen in group 4 was harvested immediately after being euthanized following a protocol that involved multiple intubations. This animal’s airway had been intubated 5 to 10 times within 1 hour. Immediate OCT imaging of the subglottis was

Figure 4. Subglottic tissue, with gray oval inset illustrating a region of interest (A). The surface boundaries of optical coherence tomography (OCT) imaging are represented by a heavy white line. Microneedles (solid black arrows) were used to mark this region. Longitudinal planes of the imaged tissue are represented by dashed lines. B. Next, the tissue was sectioned longitudinally (horizontal solid lines on each side show the cross-sectional cut sections) to allow for accurate correlation of the cross-sectional OCT image and submitted for histologic evaluation. The dotted section indicates the cross section of cricoid cartilage; the wavy red line represents the lamina propria.

Figure 5. Cross-sectional view of a native rabbit subglottis as seen on optical coherence tomography (A) and conventional histologic evaluation (B) (hematoxylin-eosin, original magnification ×40). The cricoid (CRC), first tracheal ring (TC), epithelium (EPI), basement membrane (BM), lamina propria (LP), and perichondrium (PRC) are identified.
performed. Grossly, the diameter of the subglottic region appeared reduced, and the tissue appeared edematous. This specimen represents a model of subglottic trauma that occurs following repeated or prolonged endotracheal intubation.

**OCT IMAGING**

The OCT probe was positioned over the cricoid cartilage. Figure 3 displays the OCT stage. Specimens were imaged vertically in a cephalocaudal dimension. In a configuration similar to that of a Michelson interferometer, a low-coherence light source (AFC BT1310; JDS Uniphase, San Jose, Calif) with a central wavelength of 1310 nm and a full width at half maximum of 80 nm was separated into a reference arm and a sample arm by a beam splitter. A carrier frequency of 833 kHz was generated using a phase modulator in the reference arm. The path length of the reference light is varied using a galvanometer mirror operating at 624 Hz. Light sent to the sample arm is focused onto the subglottis using a lens and then reflected back along the same pathway. The back-reflected and backscattered light of the sample is recombined with light from the reference arm and registered with a photodetector. The resultant interference fringe intensity signal is then digitized, displayed, and later transferred to a computer workstation for analysis. Two-dimensional images are formed by lateral movement of the device at a constant velocity (2.0 mm/s) and repeated after each image. Samples are acquired at a rate of 1 frame per second. The image intensity is proportional to the reflectivity of light in a given region of interest. Cross-sectional OCT images were displayed using software visualization utilities (AVS, Waltham, Mass) on a UNIX and Windows workstation platform and visualized in gray scale. The OCT imaging system and scanning stage were controlled from a personal computer workstation. The lateral and axial resolution of the system is approximately 10 µm per pixel. The image size was set laterally from 6 to 8 mm in length, as detailed images of tissue microstructures were taken up to a depth of up to 3 mm, depending on the turbidity of the media.

**HISTOLOGIC EVALUATION**

The vertical segment, imaged with the OCT probe, was marked using microneedles (Figure 4). This allows precise histologic correlation with the OCT images. Specimens were then fixed in formalin and prepared for histologic evaluation (with hematoxylin-eosin stain).
RESULTS

The epithelium, basement membrane, lamina propria, perichondrium, and cartilage (cricoid and tracheal) were clearly imaged in each of the specimens. Figure 5 illustrates OCT imaging and histologic features of the native subglottis.

In group 2, regions of collagen injection appear thickened in comparison with the native tissue sample (Figure 6). Furthermore, the signal intensity and uniformity of the lamina propria are markedly different compared with group 1. This experiment demonstrates the signal properties of the simulated scar tissue lesion and the ease with which this model can be distinguished from native samples.

Findings observed after dehydration in group 3, compared with the normal subglottis, reveal a reduction in the relative thickness of the mucosal and submucosal tissue layers (Figure 7). Rehydration of the same specimen with distilled water for 25 minutes, to simulate tissue edema, resulted in a marked increase in the thickness of the lamina propria (Figure 8).

In group 4, multiple airway intubations had produced regional tissue edema, which was seen as a wavy heterogeneous thickening of the lamina propria (Figure 9). Edema produced by intubation trauma or with hypotonic water immersion was easily differentiated from native and collagen-injected tissues.

The histologic images shown in Figure 5 through Figure 9 illustrate the submucosal microanatomy. The histologic cross-sectional images correlate with the OCT cross-sectional images, confirming the findings.

COMMENT

The subglottic airway is the open choke point for airway resistance in the neonate and infant. In addition to being the narrowest point of the pediatric airway, it is the only complete circumferential ring in the upper airway, rendering it unyielding to potential pressure caused by an endotracheal tube. The delicate pseudostratified columnar respiratory epithelium lining the subglottis is susceptible to trauma induced by the endotracheal tube. In addition, the submucosa of the subglottic region is composed of loose areolar tissue that can rapidly become edematous. With the susceptibility of these tissues to trauma, inflammation, and scar formation, the reactive capacity leading to the development of subglottic stenosis is considerable.

The findings obtained in this investigation confirm the feasibility of OCT imaging in differentiating between simulated, but distinctive, submucosal lesions in the ex vivo rabbit subglottis. Tissue edema was easily differentiated from native and collagen-injected specimens, as the signal intensity and character were specific to each structure studied. Current noninvasive imaging modalities have yet to achieve equivalent resolution and comparable depth in the imaging of these tissue microstructures.

The existence of a viable technique to diagnose and differentiate between edema, granulation tissue, scar tissue, and neocartilage formation could significantly affect the management of subglottic stenosis and acquired laryngotracheal disorders by helping determine the threshold and timing for tracheotomy or laryngotracheal reconstruction. This concept is substantiated by evidence that the severity of subglottic stenosis is dependent on the depth of injury.1,7 With such detailed tissue information, physicians would have the opportunity to more accurately designate points of surgical intervention and take preventive action in the care of their patients.

Central to the value of OCT technology is the ease in its adaptability to current endoscopic applications. The complex imaging activities of light projection, recovery, and processing are performed with the use of simple fiber-optic cables. Such technology is optimally suited for bronchoscopy of the pediatric airway. Although images in this ex vivo study were taken using a benchtop OCT device, this modality has been adapted to image tissue through a flexible OCT probe that can be passed to distant sites. Similar systems are being used at our institutions for in vivo imaging of the adult upper aerodigestive tract and related pathologic conditions. As a result of the imaging capabilities of this novel technology, as shown in this ex vivo pi-
lot study, a device is being developed to allow real-time in vivo imaging of the pediatric subglottis in the intensive care unit setting via simple insertion of an OCT imaging probe through the lumen of an endotracheal tube of a previously intubated infant (Figure 10). The information derived from high-resolution imaging and evaluation of the submucosal microstructures over time may elucidate the natural history of acquired subglottic stenosis. How this information will affect clinical decision making will require the careful evaluation of forthcoming human data. The potential use of this technology for imaging and management of the neonatal airway in the intensive care unit setting is a promising application for OCT.

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