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A Targeted Problem and Its Solution

Endoscopic Human Olfactory Biopsy Technique: A Preliminary Report

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INTRODUCTION

The histopathological changes which accompany disorders of the sense of smell are, in general, poorly understood. Although considerable understanding of the nature of such changes has recently developed, continued refinement of procedures for biopsying human olfactory epithelium (OE) is needed.

Obtaining fresh human OE for tissue analysis has been suggested through a variety of approaches with varied instrumentation. By virtue of its anatomic location, the OE is not readily visible on routine nasal examination. Difficulty in obtaining biopsy samples has been attributed to the relatively small and varied location of the olfactory area in humans. As many as six specimens may be required from each subject in order to obtain one specimen that contains OE. The presence of OE in a specimen is not, in itself, sufficient to permit study of the tissue by electron microscopy. Therefore, the technique and instrumentation applied must be capable of removing segments of OE which are intact and undistorted by the trauma of the biopsy procedure.

Several methods for harvesting this tissue for study have been reported. Biopsies performed under general anesthesia with the operating microscope, with topical anesthesia in supine position, or without any anesthesia at all have been reported. The instrumentation that has been used includes nasal forceps, toothed dissecting forceps, Nakano's forceps now available through Keheller Instrument Company, and a hook-shaped olfactory biopsy instrument (OBI).

The OBI is commercially available in the United States through the Storz Instrument Co., and was specifically designed for the biopsy of human OE. OE on biopsy specimens obtained with this instrument are meant to be free of crush artifact and therefore are satisfactory for electron microscopic (EM) analysis (Figs. 1 and 2). The technique which uses this instrumentation is routinely performed with magnification but without direct visualization of the superior turbinate. This currently represents one of the most popular techniques for the purpose of harvesting human olfactory mucosa.

The purpose of this paper is to compare the endoscopic approach using widely available instrumentation for the biopsy of human OE (Figs. 1 and 2) i.e., the 3-mm, 70-degree upturned, vertical opening, cupped giraffe forceps (Karl Storz of America) to the biopsy of OE using the OBI. The biopsy success rate using this endoscopic approach is compared to previous reports using the aforementioned "blind" technique. The OBI and giraffe instrumentation are assessed by the quality of the specimen harvested with each instrument. Tissue samples were evaluated by EM and immunohistochemical techniques. Electron microscopic and immunohistochemical studies are two very different approaches that give a variety of information regarding the ultrastructure and molecular composition of OE.

MATERIALS AND METHODS

Study subjects were recruited during a concurrent investigation of human OE from patients with early Parkinson's disease and early Alzheimer's disease. This investigation was approved by the University of Pennsylvania's
Committee for the study of Human Beings. Patients were excluded from the study if they had active rhinosinusitis, significant cardiovascular disease, history of bleeding disorder, or recent ingestion of any agent which would affect their bleeding parameters.

Endoscopically guided tissue sampling from olfactory clefts was first performed in fresh human cadavers. Once proper tissue sampling techniques were established, living subjects were enrolled for study. Informed consent was obtained and subject’s noses were topically anesthetized with 0.5% ephedrine sulfate and 2% pontocaine nasal sprays using atomizers. Further anesthesia was achieved by applying 4% cocaine solution on cotton-tipped nasal applicators to the region. Subsequently, bilateral nasal endoscopy was performed with the patient sitting upright using a 2.7-mm 30-degree nasal endoscope. Preliminary endoscopy served to identify the presence of any infection and determine which side of the nose would favor the best access to the olfactory cleft.

The superior turbinate was routinely seen with the endoscope. Upon identification of the olfactory cleft, the instrument for biopsy was introduced. The OBI was brought to the skull base between the superior turbinate and nasal septum. It was turned so that its biting surface faced the septum and was then withdrawn from the olfactory cleft. More tissue was harvested endoscopically from the same olfactory cleft using giraffe forceps. Again the olfactory cleft was endoscopically targeted and the 70-degree, forward-biting giraffe forceps were gently introduced until they met the skull base. They were then slightly withdrawn and rotated towards the septal side of the olfactory cleft. This motion occasionally resulted in out-fracture of the superior turbinate. The forceps were then opened and closed, and a bite of tissue removed. All biopsies were performed by one surgeon (D.C.L.). Although the olfactory cleft was identified each time a biopsy was taken, the actual biopsy site was not usually directly visualized during the biopsy procedure.

Once a specimen was harvested it was immediately and gently removed from the OBI or giraffe forceps and placed in the appropriate fixative for histopathologic study. Tissue was then processed for study by either electron microscopy or immunohistochemistry. Tissue samples were scrutinized for the presence of OE by light microscopy using immunohistochemical markers that discriminate OE from respiratory epithelium as described earlier. The determination of the quality of the specimen for EM evaluation was based on the presence of OE which was not distorted by traumatic artifact.

All subjects were told to anticipate intermittent bleeding from their nose for 24 to 36 hours and to take Tylenol® for any discomfort they might have. They were also instructed not to blow their nose, and to avoid any bending, lifting, or straining for the same time period.
Fig. 3. **Top left.** Region of epithelium displaying morphological characteristics of respiratory epithelium. The tissue sections were processed for immunohistochemistry as described previously.6 (H&E, original magnification × 300.) **Top right.** N-CAM staining (with MOC1 monoclonal antibody) of an adjacent section of the same region as in top left demonstrating the absence of MOC1 immunoreactivity in respiratory epithelium. Note that nerves in the lamina propria are N-CAM positive (arrow). (Original magnification × 300.) **Bottom left.** H&E-stained region of epithelium from the same sections as in top left displays morphological characteristics of OE. **Bottom right.** N-CAM staining (with MOC1 monoclonal antibody) of an adjacent section of the same region as in C demonstrating positive immunoreactivity of olfactory neurons and basal cells with MOC1. Note that olfactory nerves (arrows) and peripheral nerves (open arrow) in the lamina propria are N-CAM positive.
Thirty-seven specimens were harvested for light microscopy/electron microscopy (LM/EM) and 13 for immunohistochemical evaluation. Thus far, all of the specimens for immunohistochemical study have been reviewed and 22 of 37 specimens for LM/EM have undergone evaluation. Of the 35 specimens studied, 20 contained OE. OE was positively identified in 15 of 17 subjects.

Seven of the first group of 18 specimens harvested contained OE, whereas 13 of the last group of 17 specimens studied thus far, contained OE. Although OE was present in the earlier specimens biopsied with both the OBI and giraffe forceps, distortion seen under EM indicated these were of poor quality. Those specimens which were evaluated by EM in the last 17 specimens indicated no evidence of distortion and were considered to be of excellent quality for further ultrastructural evaluation. All but one of these was obtained with the giraffe forceps.

Analysis of OE specimens with a monoclonal antibody (designated MOC1) to neural cell adhesion molecules (N-CAM) confirmed a previous postmortem study demonstrating that the presence of N-CAMs in OE serves to distinguish OE from respiratory epithelium by immunohistochemistry (Fig. 3). In elderly individuals, atrophy, inflammation, and metaplasia may distort the morphology of the OE, making the distinction of OE from respiratory epithelium difficult. Although both types of epithelium express some of the same polypeptides (e.g., keratin and vimentin proteins), N-CAMS (as well as other proteins such as olfactory marker protein and microtubule-associated proteins) are expressed by OE and neurons but they are not expressed in respiratory epithelium. Hence, the MOC1 antibody to N-CAMS was useful in confirming the presence of OE in the biopsy samples examined by immunohistochemistry. Ultrastructural changes detected under EM in this study group will be reviewed elsewhere (Fig. 4).

**RESULTS**

Seventeen subjects fulfilled the criteria for study and underwent a total of 50 biopsies. It was found that, in the present surgeon's hands, with regard to the instrumentation, the giraffe forceps were easier to manipulate than was the OBI. Biopsies harvested with the OBI sometimes slipped from the instrument (e.g., in cases where they remained tethered to a strand of epithelium). The biting end of the cupped giraffe forceps prevented this difficulty.

A slight amount of bleeding was observed from all biopsy sites, but none required immediate special attention. Two patients phoned within 1 week of biopsy with complaints of returned bleeding. One of these patients required the placement of a tiny dissolvable Gelfoam® sponge adjacent to the biopsy site on postbiopsy day 5. The bleeding in both of these patients was attributed to postbiopsy aspirin ingestion and strenuous activity during this time period. One patient had a brief vasovagal event immediately after the specimens were obtained.

**DISCUSSION**

The olfactory neuroepithelium is situated along the roof of the nose overlying the cribriform plate and extends down onto the superior aspects of the septum and superior turbinates. Receptor cells within the olfactory cleft are the only neurons of the human body which are known to be in direct contact with the environment. They transmit signals centrally via olfactory filia. These bundles of unmyelinated nerve fibers traverse through the cribriform plate to synapse within the glomeruli of the olfactory bulb. Olfactory filia can be encased by dura and can potentially serve as conduits for centrally transmitted infections. These features of this epithelium and our general lack of knowledge regarding disorders of smell mandate the need for improved understanding of olfactory neuroepithelium.

By virtue of its anatomic location, direct access to
the olfactory cleft is limited. Similarly, this limitation is an obstacle to routine successful biopsy of OE. Perhaps one of the most popular approaches for obtaining human OE has been with a blind approach with the OBI. The biopsy success rate with the blind approach with OBI has been reported between 1:4 to 1:6 biopsies. Recent personal communication with these authors suggests this success rate is now significantly improved.

A “blind” approach was not used in this study because direct access to the olfactory cleft was ensured and not hindered by endoscopically identifying the superior turbinate. Preliminary results from this study demonstrate that the success for obtaining OE is significantly better than 1:4 specimens. The quality of specimens evaluated with EM was maintained regardless of the instrumentation used.

A comparison of the physical aspects of the instruments evaluated in this study may explain their relative efficacy. The OBI is 120 mm long, 1 mm in diameter, and its cutting edge is bent 150 degrees in a “U” shape towards the shaft. The trough created by this “U” is 1.5 mm wide, 0.5 mm deep, and 1 mm long. The narrow 1-mm shaft of the OBI had a tendency to slip and rotate between the surgeon’s fingers during use. During rotation the sharp-cutting free edge of the OBI would appear, in some cases, to be unnecessarily traumatic. The giraffe forceps is significantly larger, with a 3-mm round biting cup at its distal end. By virtue of its design, positioning the giraffe forceps within the nose can be performed with greater control than with the OBI (Figs. 1 and 2). Since the giraffe forceps are widely available to the skilled rhinologist experienced in endoscopic sinus surgery, it is a logical alternative for obtaining OE.

CONCLUSIONS

Based upon our preliminary results several conclusions can be drawn:

1. The biopsy success rate of OE is significantly improved when performed under endoscopic guidance (2:3.5).
2. A significant learning curve exists for the successful harvesting of undistorted OE for EM regardless of the instrumentation used.
3. Qualitatively similar biopsies can be harvested with either the OBI or giraffe forceps.
4. Endoscopic human olfactory biopsy can be safely performed with the giraffe forceps.
5. Further histopathological and immunohistochemical studies of OE are indicated to improve our understanding of disorders of smell.

BIBLIOGRAPHY