Chorioretinal Topography and Histopathology in Laser-Induced Choroidal Neovascularization

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Objective: To investigate the correspondence between topographic mapping of the vitreoretinal and chorioretinal surfaces in vivo and histopathology findings.

Materials and Methods: Choroidal neovascularization was induced in the retina of a primate by an argon laser. Serial optical section images of the retina were obtained using an optical imaging system based on the Retinal Thickness Analyzer. Topography of the vitreoretinal and chorioretinal surfaces was mapped. The animal was killed and the eyes enucleated for histopathologic examination.

Results: In the normal retina, the topography of the vitreoretinal surface showed a depression at the center of the fovea while the chorioretinal surface was relatively flat, corresponding to normal anatomy. In the retina with choroidal neovascularization, the topography of the vitreoretinal surface indicated a smooth elevation while there were irregular elevations in the topography of the chorioretinal surface. Histological sections displayed focal serous retinal detachment, metaplasia of retinal pigment epithelium, and choroidal neovascularization.

Conclusion: Topographic mapping of the vitreoretinal and chorioretinal surfaces in vivo corresponds with histological findings and shows promise for quantitative evaluation of pathologic alterations caused by choriotretinal diseases.


Introduction

Clinicopathologic correlation is vital to our understanding of the pathophysiology of retinal diseases. Direct visualization of the retina, most commonly using slit-lamp biomicroscopy, allows retinal diseases to be diagnosed and monitored clinically. Light microscopic evaluation of postmortem tissues permits the morphologic analysis of structural changes at the cellular level. A number of optical imaging techniques have become available that enhance the ability of ophthalmologists to visualize retinal structures in the clinical setting.

These techniques have made possible noninvasive, objective, and quantitative evaluation of the retina in the normal state as well as detection of abnormalities caused by disease and injury. Scanning laser ophthalmoscopy (SLO) has been used to map the topography of the retinal surface. 

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Optical coherence tomography (OCT) provides high-resolution cross-sectional images of the retina and quantitative measurements of retinal thickness. The Retinal Thickness Analyzer (RTA; Talia Technologies Ltd, Neve Ilan, Israel) allows mapping of the retinal thickness.

In this report, we used a prototype RTA to generate optical section images of the retina in vivo and map the topography of the vitreoretinal and chorioretinal surfaces in primate eyes. The optical section retinal images obtained in a normal eye and in an eye with laser-induced choroidal neovascularization were compared to histopathologic sections of the retina.

MATERIALS AND METHODS

Instrumentation

The instrument for retinal topography and thickness mapping is based on the optics of a slit-lamp biomicroscope and has been described in detail previously. In vivo imaging consisted of generating serial optical section images using an obliquely projected scanning narrow laser slit. The laser beam was scanned on the retina to generate 20 optical section images encompassing a 2×2-mm retinal area in 0.33 seconds.

The optical section images were analyzed to generate topographic maps of the vitreoretinal and the chorioretinal surfaces. A dedicated software program analyzed the digital optical section images and detected the location of the two reflection intensity peaks corresponding to the vitreoretinal and chorioretinal interfaces by a curve-fitting algorithm. Because the analysis algorithm detects the peak locations corresponding to the retinal interfaces, changes in the reflection and scatter of light that result in broadening of the lines do not significantly affect measurements.

The analysis was repeated along the vertical axis of all 20 optical section images. The relative heights of the vitreoretinal and the chorioretinal interfaces were determined according to a fixed reference and stored in two 2-dimensional arrays. The data in the arrays were smoothed in both dimensions by filtering and scaled by setting the minimum height in the imaged area as baseline. Three-dimensional topography maps of the vitreoretinal and chorioretinal surfaces were plotted. For viewing the optical section images, a composite image was created that consisted of 10 optical sections of the retina, each spatially separated by 200 mm, displaying a 2×2-mm retinal area.

Animals

All procedures involving animals were performed according to the institutional guidelines established by the University of Illinois at Chicago and followed the Association for Research in Vision and Ophthalmology (ARVO) statement on the Use of Animals in Vision Research. The experiment was performed in a baboon. General anesthesia was administered with intubation and intravenous anesthesia (sodium pentobarbital) was administered during the procedure. In one eye, laser lesions were created by applying an argon laser (P=700 mW, ω=100 mm, τ=100 msec) to create a model for subretinal neovascularization. Fluorescein angiography was performed, and the findings were consistent with the presence of choroidal neovascularization.

Optical section retinal imaging was performed 4 weeks following laser photoagulation. The eye was dilated (10% phenylephrine and 1% tropicamide), and local anesthetic drops (0.5% proparacaine) were applied. The eye was fitted with a contact lens to prevent corneal dehydration. Optical section images were generated by scanning a laser slit on the retina. The baboon was then sacrificed; both eyes were enucleated and submitted for histopathologic processing. For histologic sectioning, the retina was fixed in 4% paraformaldehyde and 1% glutaraldehyde, postfixed in Dalton's osmium fixative, dehydrated in alcohol, and embedded in epoxy resin. One micrometer serial sections were cut in the area surrounding and encompassing the lesion.

RESULTS

Normal Retina

Optical section images were generated in the untreated primate eye and analyzed to map the topography of the vitreoretinal and chorioretinal surfaces. A
Figure 2. A composite image displaying the series of 10 of 20 optical sections generated by scanning the laser across the foveal area separated by 200 μm on the retina. The right and left arrows point to the reflection and scattering of the laser from vitreoretinal and chorioretinal interfaces, respectively. The arrowhead points to the center of the fovea. The histologic sectioning in a normal baboon eye indicates depression of the retinal surface at the center of the fovea (original magnification ×10) (methylene blue). The histologic section corresponds to the optical section marked by the arrowhead.

box superimposed on the fundus photograph and fluorescein angiogram (Figure 1) indicates the area of imaging. By scanning the laser across the foveal area, a series of 20 optical sections separated by 100 μm on the retina were generated. A composite image was created that displayed 10 of the series of 20 optical sections separated by 200 μm on the retina (Figure 2). Each optical section image consisted of the reflection and scattering of the laser light from the vitreoretinal and chorioretinal interfaces. The center of the foveal depression was visualized as the point with minimum separation between the two retinal interfaces. Topography of the vitreoretinal surface displayed a depression at the center of the fovea corresponding to normal anatomy. In contrast, topography of the chorioretinal surface had a relatively flat appearance (Figure 3). Histologic sections of the normal eye indicated a similar depression of the retinal surface at the center of the fovea (Figure 2).

Laser-Treated Retina

Optical section imaging and topographic mapping were performed in the baboon eye that underwent experimentally induced choroidal neovascularization. A box superimposed on the fundus photograph and fluorescein angiogram (Figure 4) indicates the area of imaging. Fluorescein angiography showed late leakage consistent with the presence of choroidal neovascularization. The optical section images displayed a significant elevation of the vitreoretinal interface (Figure 5). A bright reflex appeared on several optical section images because of the reflection of the laser light from the internal limiting membrane, often highly visible in young primate eyes. The retinal blood vessels appeared as dark lines between the vitreoretinal and chorioretinal interfaces. Topography of the vitreoretinal surface indicated a smooth elevation while topography of the chorioretinal surface displayed localized and irregular elevations (Figure 6). Histologic sections from the same retinal area displayed a focal serous retinal detachment, metaplasia

Figure 3. Topographic map of the vitreoretinal surface displayed a depression at the center of the fovea while the chorioretinal surface appeared relatively flat.

Figure 4. (A) Fundus photograph and (B) fluorescein angiogram of a left baboon eye with experimental choroidal neovascularization. The area of imaging is outlined by the box.

OPHTHALMIC SURGERY, LASERS & IMAGING · JANUARY/FEBRUARY 2003 · VOL 34, NO 1
Figure 5. A series of optical section images demonstrate significant elevation of the vitreoretinal interface. A bright reflex (arrow) appeared caused by the highly reflective inner limiting membrane in young primates eyes. The retinal blood vessels appeared as dark lines (arrowhead) between the vitreoretinal and chorioretinal interfaces. The histologic section, corresponding to the optical section second from the left of the series of optical sections, displayed a serous retinal detachment and metaplasia of the retinal pigment epithelium (original magnification ×10) (methylene blue).

of the retinal pigment epithelium, and elevation of the retinal surface (Figure 5). Choroidal neovascularization was observed under higher magnification (Figure 7).

**DISCUSSION**

Age-related macular degeneration is a leading cause of irreversible visual loss in the elderly in the western world.27-30 With choroidal neovascularization causing significant visual loss in age-related macular degeneration.31 A number of histopathologic studies have contributed to our understanding of the pathophysiology of choroidal neovascularization in age-related macular degeneration.32-34 These studies have reported ingrowth of choroidal blood vessels within and through defects in Bruch’s membrane along with proliferation of other cells including fibroblast, retinal pigment epithelium, and inflammatory cells.

Although considered the gold standard in the evaluation of morphologic changes at the cellular level, light microscopic analysis of fixed tissues have several disadvantages and limitations. Tissues must be surgically excised before evaluation can be performed. The processing of the specimen introduces potential artifacts to the retinal morphology. Surgical excision of retinal tissue may have the potential for complications to the retina and its surrounding tissues. It can be used only for the evaluation of macular diseases in selective cases.

Figure 6. Topographic map of the vitreoretinal surface displayed a smooth elevation while the chorioretinal surface presented irregular elevations.

A few studies have compared OCT cross-sectional images with histology, both in the normal and pathologic states.35-38 In the present study, our optical sectioning imaging system was applied to the normal pri-

Figure 7. Higher magnification of the histologic section displayed in Figure 5 (original magnification ×40) (methylene blue). Choroidal neovascularization is noted with red blood cells present in the lumens (asterisks). The irregular surface of the neovascular membrane can be seen.
mate retina as well as a retina afflicted with induced choroidal neovascularization. The capability of our system for imaging the retinal interfaces, mapping the retinal topography, and identifying topographic changes at the vitreoretinal and chorioretinal surfaces was demonstrated. In addition, a correspondence between retinal optical section imaging and histology for the detection of topographic alterations in the vitreoretinal and chorioretinal interfaces was established.

The topography of the chorioretinal surface is altered in a number of diseases, and it may be difficult to assess the changes by direct clinical observation. The presence of classic choroidal neovascularization is identified by leakage on fluorescein angiography. However, in most cases, an occult component of the lesion is also present and angiography may be inadequate for detection of the entire extent of the neovascularization.

Because the presence of subretinal fluid and lipid and subretinal pigment epithelial fibrovascular membrane have been shown to be associated with occult choroidal neovascularization, topographic assessment of the chorioretinal surface, along with conventional angiography, may improve the evaluation of this condition. Optical coherence tomography allows visualization of intraretinal structures because of its higher-depth resolution.

Our chorioretinal optical sectioning system, as well as OCT, has adequate depth resolution for imaging the chorioretinal interface. Additionally, our system provides topographic maps of a large retinal area in a short time (2×2 mm area in 1/3 of a second) with a high spatial resolution (100 μm in both directions). Overall, our system offers a noninvasive means of quantitatively and reliably evaluating the chorioretinal interface and shows promise for diagnosis and monitoring of pathologic lesions caused by chorioretinal diseases.

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REFERENCES


