Noninvasive Imaging by Optical Coherence Tomography to Monitor Retinal Degeneration in the Mouse

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PURPOSE. Optical coherence tomography (OCT) is a high-resolution imaging technique that measures the intensity of backscattered light from biological microstructures in living tissue. The objective was to evaluate OCT as a routine, noninvasive technique for quantitative measurements of retinal thickness and detachment in small animal models of retinal degenerative diseases.

METHODS. An OCT scanning unit was designed and built to visualize retinal tissue from rodents at high resolution in vivo. Several normal and retinal degeneration (rd) mouse strains with different pigmentation, as well as a transgenic mouse strain that carries a wild-type β-PDE gene in an rd/rd background, were analyzed at different ages. Retinal detachment was induced by subretinal injection of saline. Retinal function was evaluated by full-field ERG, and then each retina was cross-sectionally scanned by OCT. OCT image analysis and measurements of retinal thickness were performed. Animals were then killed and retinal histology was documented.

RESULTS. OCT images of the mouse retina revealed structural landmarks allowing assignment of retinal structures. There was no difference in the OCT pattern between pigmented and nonpigmented mice. Changes in the retinal thickness measured by OCT correlated very well with the loss in function measured by ERG and histology in rd/rd and rd/rdtg transgenic mice at a variety of ages. In addition, retinal detachment caused by surgery was easily visualized and observed by OCT imaging.

CONCLUSIONS. OCT imaging is applicable to the mouse retina. There is excellent agreement between the retinal thickness measured by OCT, ERG amplitude, and retinal histology, thus validating OCT imaging as a sensitive and noninvasive tool for monitoring the structural progression of retinal diseases in rodent models. OCT also appears useful for visualizing retinal detachments in the mouse. (Invest Ophthalmol Vis Sci. 2001; 42:2981–2989)

Rodent models are extremely useful in understanding the cellular and molecular events associated with retinal degenerative diseases.1,2 Unfortunately, it has been difficult to monitor the cellular structure of the rodent retina without killing the animal for conventional histology. Consequently, large numbers of genetically defined animals must be used to obtain a convincing experimental result. The pivotal issue is that retinal morphology cannot be observed noninvasively through the course of degeneration in a single animal; thus, each animal contributes just a single time point to understanding a full disease progression. Given the variety of new gene and pharmacologic therapies that may retard the progression of retinal degeneration,3–5 the ability to observe a therapeutic regimen through its entire course in individual rodent models would greatly enhance the speed and reliability of testing and would reduce the number of experimental animals required. The growing number of newly recognized mouse models exhibiting genetically based retinal degeneration6–9 further emphasizes the need for rapid, noninvasive analysis of retinal degeneration in small eyes.

Optical coherence tomography (OCT) is a noncontact, noninvasive optical imaging technique that measures the intensity of backscattered light.10–12 OCT was originally developed for performing high-resolution tomographic imaging of ocular tissues, but has recently been used for imaging a wide range of nontransparent tissues as well.13–17 OCT produces cross-sectional images of optical reflectivity in the tissue analogous to ultrasound B-scan, providing greater resolution by using light instead of sound waves. The principle of OCT is based on low-coherence interferometry. A schematic of the apparatus is shown in Figure 1. OCT is now relatively common in clinical imaging of the human eye for diagnostic purposes.18 It has also been used for imaging animals with relatively large eyes such as chicken and swine.19,20 However, it has not been applied to small animal models of retinal disease. In this study, we tested a new OCT technology for imaging the mouse retina and correlated it with conventional ERG and histology studies, to develop a regimen for OCT imaging of animal models of retinal degenerative diseases. To validate this methodology we also documented an OCT-histology-ERG correlation occurring during degeneration of the retina in the transgenic rd/rdtg mouse containing a normal β-PDE transgene in the rd/rd mouse background.20 In addition, we tested OCT to detect retinal detachments induced by subretinal saline injections, to test whether the technique could monitor the success of intraocular injections and subsequent recovery from the initial subretinal bleb.

MATERIALS AND METHODS

Animals and Injections

These studies adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, as well as local government regulations for the use of animals in ophthalmic and vision research. Mouse strains C57BL/6J (black), BALB/Cj (albino), and C3.B1Ba (agouti), and two rd mouse strains, C3H/HeJ (agouti) and SJL/J (albino) were obtained from Jackson Laboratories (Bar Harbor, ME). An rd/rd/
length of two arms is matched within the coherence length of the light source. By changing the distance of the reference arm, reflected signals from different depths within the tissue are combined and detected, whereas light from other points within the tissue does not interfere at the detector.

$tg^-$ transgenic strain$^{20}$ was recovered from frozen embryos (a generous gift of Janis Lem and Melvin Simon). Phosphate-buffered saline (PBS, 1 μl) was injected into the subretinal space of each eye with a blunt 33-gauge needle through a 28-gauge opening in the peripheral cornea under a dissecting microscope.$^{21}$

**ERG Analysis**

ERG recordings were performed using a visual electrodiagnostic system (UTAS-E2000; LKC Technologies, Gaithersburg, MD). Animals were dark adapted overnight and anesthetized before the experiment with an intraperitoneal injection of a saline solution containing ketamine (18 mg/kg body weight) and xylazine (3 mg/kg body weight). Eyes were dilated in dim red light with 2.5% phenylephrine solution. Small contact lenses with gold wire loops$^{22}$ were placed on each cornea with a drop of 2.5% methylcellulose to maintain corneal hydration. A silver wire reference electrode was placed subcutaneously (SC) between the eyes, and a ground electrode was placed SC in a hind leg. Full-field scotopic ERGs were elicited with 10-μsec flashes of white light from 0.447 log candelas (cd)/sec · m² to –2.8 log cd/sec · m². Responses were amplified at a gain of 4000, filtered between 0.3 and 500 Hz and digitized at a rate of 2000 Hz on two channels. Three responses were averaged at each intensity. The a-waves were measured from the baseline to the peak in the cornea-negative direction, and b-waves were measured from the cornea-negative peak to the major cornea-positive peak.

**OCT Scanner**

The implementation of OCT was achieved using a compact, fiberoptic-based OCT scanner. A superluminescent diode operating at 1300-nm superluminescent diode is coupled into a fiber interferometer and is split by a 50-50 fiber splitter into reference and measurement beams. The measurement beam is reflected from internal structures within the tissue, travels back through the sample arm and reaches the detector. Light sent into the reference arm is collimated at the output of the reference arm fiber by a lens, reflected from a mirror, refocused by the lens, and recombined with the sample arm beam. Constructive interference (thus a signal) is observed only when the optical path length of two arms is matched within the coherence length of the light source. By changing the distance of the reference arm, reflected signals from different depths within the tissue are combined and detected, whereas light from other points within the tissue does not interfere at the detector.

**OCT Imaging and Analysis**

Before OCT imaging was performed, each animal was anesthetized and the pupils dilated. Because of the small eyes (3 mm diameter) and the lack of landmarks in the mouse retina, OCT scanning was initially directed to the center (posterior pole) of the mouse eye, facilitated by the aiming beam of the scanner. The scanning head of the OCT unit was connected to a device that allowed two-dimensional movement. The anesthetized mouse was placed in an adjustable holder that could be rotated easily. For horizontal scan, the aiming laser was directed to the center of the mouse eye in alignment with the lateral nasal-to-temple raphe and vertical scan with superior-to-inferior across the center of the eye. Each scan was performed at least twice, with realignment each time. The dimension of the scan (in depth and transverse extent) was adjusted until the optimal signal intensity and contrast was achieved. Retinal thickness was measured from the central retinal area of all images obtained from both horizontal and vertical scans from the same eye, using the system software, and averaged.

The method used to extract the retinal thicknesses in the system software was based on the fact that the photocurrent from the detection photodiode was amplified in a logarithmic detector and digitized with 12-bit resolution. Thus, each pixel on the OCT B-scans represented one of 4096 possible levels. Once acquired, the B-scans were linearly filtered (9-pixel averaging). Areas of interest (AOIs) were established based on visual inspection of the retinal layers in the B-scan. In the AOIs, lineouts were taken of the digitized OCT B-scans, recording pixel intensity versus position (depth). Boundaries between differing layers were established by directly measuring the widths of features by determining the positions where the pixel intensity reached $I_{max} - (I_{max} + I_{max})/2$, where $I_{max}$ are the average values of the low and high intensity features. The group index used to convert to physical path was 1.38. (The value for water is 1.34, which is probably slightly less than the retina, 1.376 for cornea, 1.41 for lens, and 1.356 for aqueous humor and vitreous humor.)

**Histology and Retinal Thickness Measurements**

Histologic analysis of mouse retinas was performed by using standard procedures.$^{23}$ Mice were killed by CO₂ overdose and perfused intracardially with 4% paraformaldehyde and 1% glutaraldehyde fixative after the blood was washed out with saline. Eyes were fixed in the same fixative for 1 to 2 days and embedded in epoxy resin. Sections were cut at 1-μm thickness along the vertical meridian and stained with tol-
idine blue. In some cases, eyes were fixed in the same fixative for 2 to 4 hours and embedded in polyester wax (EM Science, Fort Washing-
ton, PA), after gradient ethanol dehydration and wax infiltration. Sec-
tions were cut at 8-μm thickness along the vertical meridian and
stained with hematoxylin and eosin. The retinal thickness was mea-
sured from polyester wax embedded sections using the measurement
tool of a microscope (Axioplan 2; Carl Zeiss, Oberkochen, Germany).

RESULTS

Prior work (Reitze D, unpublished results, 1998) on ocular OCT
imaging has used an 800-nm light, primarily because the aqueous-
vitreous humor is more transmissive at 800 nm than at longer
wavelengths. A preliminary comparison of OCT images obtained
with a 1280- versus 800-nm wavelength of light in primate eyes

Figure 2. (A, C) Light micrographs of retinas (top) and scotopic ERG wave forms (bottom) of 1-month-old normal (A) and rd (C) mice. The scale bar is 50 μm. The ERG waves represent the average of five measurements at maximum intensity. (B, D) OCT images of the retinas of normal (left) and rd (right) mice. The ruler scale is in millimeters. RPE, retinal pigment epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; INL, inner nuclear layer; OPL, outer plexiform layer; IPL, inner plexiform layer; GCL, ganglion cell layer.
revealed both decreased signal and resolution at longer wavelength (Reitze D, unpublished observation, 1998). Although light scattering is diminished at longer wavelengths, this advantage is offset by an overall increased absorbency of biological material at 1280 nm and an increase in coherence length owing to the spectral dependence of the absorbency. However, these problems are substantially diminished in rodent eyes, owing to their relatively small diameter and correspondingly shorter path length. Thus, we designed and built a 1280-nm OCT imaging unit to obtain high-resolution retinal structure in the living rodent.

Features of OCT Images of the Mouse Retina

To assess the sensitivity of OCT imaging in detecting differences in rodent retinal thickness and morphology, we used a normal and a retinal degeneration (rd) mouse strain. To test the effect of pigmentation on the pattern of the OCT images, we also examined three normal mouse strains, C57BL/6J (black), BALB/CJ (albino), and C3.BliA (agouti), and two rd mouse strains, C3H/HeJ (agouti) and SJL/J (albino). Each animal was first tested by ERG, then by OCT, and finally killed for retinal histology at the end of the experiment.

One of the primary applications of OCT analysis in the mouse would be to observe structural changes in animals with degenerative diseases of the retina. The rd mouse is a relatively fast-degenerating model because of a mutation in the gene coding for the β-subunit of cGMP-phosphodiesterase (β-PDE). The outer layer of the rd mouse retina undergoes severe degeneration beginning at postnatal day 8. At 1 month of age, the outer (OS) and inner segments (IS) of rod photoreceptors have nearly completely disappeared, and the outer nuclear layer (ONL) has been reduced to a single row of nuclei (compare Fig. 2). Loss of photoreceptors, as expected, correlates with the complete loss of the scotopic ERG b-wave response.

By comparing OCT and histologic images, it was possible to begin identifying the limits of the retinal layer in OCT images. OCT scan images of normal (left) and rd (right) mouse retinas are shown in Figures 2B and 2D. The backscattered light intensity is displayed in a grey-scale in which the lighter areas correspond to regions of high relative reflectivity or backscattering, and dark regions represent areas of minimal or no relative reflectivity. The first reflective interface posterior to the lens at the top of each image in Figures 2B and 2E clearly represents the anterior border of the retina at the vitreoretinal interface. Using this OCT image interface a starting point, it was possible to define the set of OCT layers corresponding to the full-thickness normal retina. To aid this assignment, we analyzed a series of normal retinas in which 1 μl PBS was injected into the subretinal space of each mouse eye near the posterior pole. Local regions of detached retina in OCT image allowed us to distinguish retina from underlying RPE-choriocapillaris (Fig. 3). The detached retina was clearly evident in all images. The highly reflective layer below the retina must represent the anterior border of the choriocapillaris-retinal pigment epithelium. The detached layers were therefore intact, full-thickness retina that had ruptured from the injection procedure (Fig. 3). By virtue of being on the posterior face of the detached retina, the photoreceptor layer could be identified as the minimally reflective layer located in the posterior side of the retina. This layer was quite evident in the intact retinas seen in Figures 2B and 2D as the nearly nonreflective layer adjacent to the RPE-choriocapillaris.

This interpretation is consistent with the assignment and appearance of the photoreceptor layer in the primate retina according to Peiroth et al. It has been reported that there are four pseudocolored bands in OCT images of human extrafoveal retina. The innermost band is thought to correlate with the retinal nerve fiber layer (RNFL) at the vitreous interface, and the outermost band is thought to be a function of the retinal pigment epithelium (RPE)-choriocapillaris complex. The middle two bands in the human retina are thought to correspond to the inner plexiform layer (IPL) and outer plexiform layer (OPL), respectively. However assigning these reflective regions to specific histologic landmarks has been controversial because of difficulties in resolving the various components of the OCT images. In the OCT images of the normal mouse retina (Figs. 2B, 4 at higher magnification), these two reported middle bands are not evident. Instead, there is one additional low-reflection region that divides the anterior part into two bands that clearly are not IPL and OPL. This entire band is substantially thinned in the images of rd mouse retina after the photoreceptor layers have been lost (Figs. 2D, 4), confirming its assignment as the combined inner and outer retina. We also compared OCT retinal images from albino and pigmented mice and found no difference in the pattern of reflective layers as a function of pigmentation (data not shown).

Quantitative OCT Analysis of a Degenerative Mouse Retina

In contrast to the rapid retinal degeneration of the rd mouse, the transgenic rd/rd/tg mouse, initially reported to be res-
cued from retinal degeneration, in fact loses retinal function over a more protracted period (John G. Flannery and Janis Lem, personal communication, 1997). We sought both to validate the application of OCT for monitoring retinal changes in the mouse and, in parallel, to document the time course of this “slow” retinal degeneration by observing photoreceptor layer thinning as the transgenic $rd/rd$/$tg$1 mouse ages. At 1 month, the retinas of $rd/rd$/$tg$1 mice have apparently normal morphology and scotopic ERG responses. We observed progressive retinal degeneration beginning at approximately 8 weeks, with reduction of the ONL to one to two rows of nuclei or less by 3 to 4 months (Fig. 5A). There was some variation among animals in the rate of degeneration, even among littermates, perhaps due to differences in transgene copy number or ambient light conditions. The relatively slow retinal degeneration in this $rd/rd$/$tg$1 transgenic mouse line compared with the $rd/rd$ mouse better mimics human retinitis pigmentosa (RP), suggesting that this transgenic mouse may be a valuable animal model for studying retinal degenerations due to mutations in the $\beta$-PDE gene and for developing therapeutic interventions.

Using the $rd/rd$/$tg$1 model, we quantitated the relationship between cross-sectional OCT images and scotopic ERG signal amplitudes. Measurements of retinal thickness were obtained directly from tomograms by measuring the distance between the inner and outer retinal boundaries. Images were not post-processed, except for filtering and smoothing to facilitate the measurements. For simple comparison among mice at different stages of retinal degeneration, the average retinal thickness in the central 1 mm of the retina was used. Horizontal and vertical scans from both eyes over this region were measured and averaged for each animal. The $rd/rd$/$tg$ transgenic mice from 4 to 16 weeks old were observed with ERG and OCT measurements. Retinal histology was also obtained at 4, 6, 8, 10, 12, 14, and 16 weeks of age after ERG and OCT measurements. The thickness of the normal mouse retina measured by OCT is 220 to 250 $\mu$m. In a 1-month-old $rd/rd$ mouse, the

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**Figure 4.** High-magnification light micrograph and OCT image of a normal (top) and 1-month-old $rd/rd$ (bottom) retina aligning with identification of the retinal layers. The OCT images were displayed in brown color scale in which the bright colors correspond to regions of high relative optical reflectivity or backscattering, and dim colors represent areas of minimal or no relative reflectivity. Note that OCT images are inverted relative to those in Figures 2 and 3. The scale of the OCT image is not same as the scale bar. See Figure 2 for abbreviations. Scale bar, 50 $\mu$m.
retinal thickness is reduced to 140 to 160 μm because of complete loss of the OS and IS of the rod photoreceptors and more than 90% thinning of the ONL by this age. Retinal thickness measured from fixed sections of the normal mice is approximately 107 ± 5 μm. All the measurements were performed from polyester-embedded sections as described in the Materials and Methods. Retinal thickness values are different from the ones seen in plastic embedded sections as shown in micrographs (Figs. 2A, 2C, 4, 5A), due to shorter fixation time and extensive tissue shrinkage during dehydration and wax infiltration process. In the adult rd/rd mouse, the thickness is reduced to approximately 55 ± 2.5 μm. In the transgenic rd/rd/tg⁺ mouse, the progressive loss of photoreceptors, and thus the thinning of the retinal layer, can be detected by OCT imaging (Fig. 5B). The time course of retinal degeneration in rd/rd/tg⁺ transgenic mice measured by full-field scotopic b-wave ERG amplitude (Fig. 6A) and retinal thickness changes measured from fixed sections (Fig. 6B) and OCT images (Fig. 6C) are shown in Figures 6A, 6B, and 6C. A comparison between the retinal thickness measured from fixed sections and OCT images is shown in Figure 6D. When normalized to the same values at 4 weeks of age, there was no statistically significant difference between the OCT thickness measurements and histologic retinal thickness at any stage of degeneration.
from 6 to 16 weeks of age (Fig. 6D). Because retinal degeneration in the rd/rd/tg1 mice is due to loss of photoreceptors, when the percentage of reduction in photoreceptor layer (OS, IS, and ONL) instead of the entire retinal thickness was calculated, there was also agreement between photoreceptor layer thickness measurements (fixed sections or OCT images) and ERG amplitude (Fig. 6E). Thus, although the fine retinal structures remained difficult to resolve in OCT images, overall changes in the retinal thickness were easily determined by OCT and reflected well the loss in retinal function measured by ERG in these transgenic mice.

**DISCUSSION**

In the current study, OCT imaging with a 1280-nm scanner was applicable to the mouse retina. The technique is noninvasive,
rapid (full retinal scans took <5 seconds each) and there was a good correlation between the retinal thickness measured by OCT and by retinal histology. In addition, losses in ERG amplitude agreed extremely well with OCT monitoring of retinal thinning in a mouse model of degenerative retinal disease.

OCT imaging has several important advantages over other imaging technologies and conventional histologic studies. First, it is noninvasive, and the same specimen can therefore be monitored over a prolonged period to observe changes in morphology in the same animal. Second, OCT is an in vivo technique, measuring tissue structures under living conditions, and it therefore does not suffer from artifactual changes in tissue morphology associated with histologic sample preparation. Third, it is sufficiently sensitive to detect relatively small changes in the retinal thickness (±20 μm). Fourth, OCT scanning and image acquisition is very fast and relatively inexpensive, and results can be obtained easily from a large number of animals within a period comparing very favorably to lengthy histologic procedures. Fifth, OCT images are digitized and therefore are inherently quantifiable for statistical analysis. Finally, OCT imaging and standard ERG analysis can be performed sequentially (ERG first) on the same animal, thus allowing essentially simultaneous documentation of retinal structure and physiology in the living animal.

There remain some disadvantages to OCT imaging of small retinas. First, the resolution of our current OCT system does not allow a direct comparison of retinal substructures with that obtained by histology. Whereas retinal histology displays definitive boundaries at high resolution between layers due to distinct cellular interfaces, such details are not as well resolved with OCT imaging at the power levels and spatial resolution currently achievable. This problem is at least partially attributable to the relatively small changes in layer-to-layer scattering coefficients. However, laser-based OCT imaging has recently demonstrated retinal imaging with less than 3-μm resolution, potentially overcoming this limitation. Second, the propagation of light within tissue is strongly affected by its scattering properties. The intensity and contrast of retinal imaging is severely attenuated by even minor corneal opacity, cataracts, and other diseases that may be present in the anterior segment of the eye. Third, although OCT images are digitized and thus quantifiable, the precise location of tissue boundaries in OCT images can be difficult to accurately define. We are currently evaluating the use of an additional lens on the cornea to improve the OCT image of this region. However, when observing retinal degeneration in animals that progresses to complete or nearly complete loss of photoreceptors, the approximately 50- to 100-μm retinal thinning that ensues can be easily and reproducibly monitored. In addition, the readily visualized retinal detachment using OCT scanning makes it an extremely useful way to detect any disease or structural alterations associated with subretinal surgical procedures, thus allowing rapid evaluation of the success of subretinal injection of therapeutic or experimental reagents.

References


