Objective: To study whether optical coherence tomography (OCT) scans correlate retinal histologic findings with the progression of retinal degeneration in retinal degeneration slow (rds) mice.

Methods: Sensory retinal thickness (SRT) and outer retinal thickness (ORT), representing photoreceptor cell layer, in temporal retina at a distance 1 to 2 disc diameters from the optic disc were measured using scan profile in OCT from 6 healthy mice (16 weeks old) and 2-week-old (n=6), 6-week-old (n=4), and 60-week-old (n=2) rds mice. Histologic sections were obtained from Epon-embedded retinas from the corresponding location.

Results: Cross-sectional OCT images correlated to the corresponding histologic sections in each mouse. Both SRT and ORT of 2-week-old rds mice (150±4 µm and 28±4 µm, respectively) lacking photoreceptor outer segments were already shorter than those of healthy mice (174±5 µm and 37±6 µm, respectively) (P<.001). In 6-week-old mice, microscopic findings revealed a decreased number of nuclei in the outer nuclear layer, and SRT and ORT (136±2 µm and 20±1 µm, respectively) were shorter than those of 2-week-old rds mice (P<.001). The SRT of 60-week-old rds mice without a photoreceptor layer was remarkably reduced (120±7 µm), and no ORT could be measured.

Conclusion: Our findings suggest a possible relationship between SRT and ORT, as measured by OCT, and histologic change in retinal degenerative diseases.

Clinical Relevance: The quantitative analysis obtained by OCT scans may have potential to detect progressive change in degenerative retina and may be used in studying human retinal degeneration.


Optical coherence tomography (OCT) provides a noninvasive, high-resolution, cross-sectional image analog to ultrasound imaging. The resolution of OCT is reportedly 10 µm with the use of low-coherence interferometry. Optical coherence tomography has been used as a diagnostic method in studying several retinal diseases, because it provides quantitative information for longitudinally tracking small changes in tissue structure and the development of disease processes. Recently, OCT has been used to study degenerative retina in humans and animals. These studies have described a thinner retina in eyes with retinal degeneration compared with healthy subjects. Optical coherence tomography may prove a valuable noninvasive technique in the study of human retinal degeneration. However, only a few studies have compared OCT scans with histologic sections, and the potential of OCT to detect progression in degenerative retina is unclear.

In retinal degeneration slow (rds) mice, the outer segments of photoreceptors fail to develop and are followed by slow degeneration. The rds mice have been studied because they presumably make a good model for some forms of hereditary retinal degeneration occurring in humans. To study the relationship between changes of OCT image and histologic progress of retinal structure in degenerative retina, we used OCT scan profiles in healthy and rds mice.

RESULTS

Cross-sectional OCT images were obtained, suggesting a close relationship with the corresponding histologic sections in normal and rds mice (Figure 3). Highly reflective red layers that delineate the surface and the posterior boundary of the retina and corresponding to NFL and RPE with...
Moreover, SRT and ORT of 6-week-old mice were statistically shorter than those of normal mice (P < .001). No relative low reflectivity layer anterior to the RPE may correspond to the retinal pigment epithelium (RPE) and choriocapillaris (Figure 1). Another highly reflective red layer in pseudocolor scale delineating the surface of the retina in OCT image may correspond to retinal nerve fiber layer (NFL), including the internal limiting membrane (ILM). Measurement of SRT and ORT was performed manually using LRP in a scan profile program of OCT (Figure 2). We demonstrated that SRT and ORT measured by OCT scan profile decreased with progression of retinal degeneration in 6-week-old mice, which had a decreased number of nuclei in the outer nuclear layer compared with 2-week-old mice. The photoreceptor layer of 60-week-old rds mice was almost gone, and SRT was 120 ± 7 µm. The SRT of 60-week-old rds mice was shorter than that of any other group of mice (P < .001). No relative low reflectivity layer was found in a cross-sectional OCT image in 60-week-old rds mice.

Comment

Histologic findings revealed the progress of retinal degeneration in 6-week-old mice, which had a decreased number of nuclei in the outer nuclear layer compared with 2-week-old mice. The photoreceptor layer of 60-week-old rds mice was almost gone, and SRT was 120 ± 7 µm. The SRT of 60-week-old rds mice was shorter than that of any other group of mice (P < .001). No relative low reflectivity layer was found in a cross-sectional OCT image in 60-week-old rds mice.

We demonstrated that SRT and ORT measured by OCT scan profile decreased with progression of retinal degeneration in rds mice and the cross-sectional OCT image cor-

MATERIALS AND METHODS

ANIMALS

Six adult (16-week-old) mice (Balb/c-57) served as normal controls, and six 2-week-old, four 6-week-old, and two 60-week-old rds mice were used in this study. All animals were maintained in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and institutional approval was obtained. The mice were anesthetized with an intraperitoneal injection of 15 µL/g of isotonic sodium chloride solution containing ketamine hydrochloride (1 mg/mL), xylazine hydrochloride (0.4 mg/mL), and urethane (40 mg/mL). The pupils were dilated with 0.5% phenylephrine hydrochloride and 0.5% tropicamide. Mice were placed on a heating pad throughout the experimental session.

OCT IMAGING

Cross-sectional imaging of the retina was performed using an OCT instrument (Humphrey Instruments, San Leandro, Calif), which has been described previously. In brief, low-coherence light (center wavelength, 850 nm) from a superluminescent diode source is guided into a fiberoptic Michelson interferometer and is divided at a fiber coupler into reference and sample paths. The reflected light from the reference mirror and the eye is recombined in the coupler. The 2 light pulses produce an interference signal at the detector only when the reference arm distance matches the length of a reflective path through the eye to within the source coherence length, which predicts a longitudinal resolution of 10 µm full width at half-maximum in the retina. This longitudinal resolution is unaffected by ocular aberrations or limited pupil diameter.

A single 1-dimensional, longitudinal profile of optical reflectivity vs distance into the tissue is created by translating the reference arm mirror and measuring the magnitude of the interference signal at the detector. A 2-dimensional, cross-sectional image is created by obtaining multiple, longitudinal reflectivity profile (LRP) and is displayed using a pseudocolor scale while rapidly scanning the probe beam through tissue. The fundus view with a visible light source placed coincident with the probe beam can be obtained by an infrared sensitive video camera.

Anesthetized mice were aligned with the OCT so that the optic disc of their right eye was visible. The OCT images were oriented horizontally, and each scan was recorded from the nasal edge of the optic disc, across the center of the optic disc, to the temporal retina. The lateral extent of scans was 2.8 mm.

HISTOLOGY

Eyecups were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde, refluxed with 1% osmium tetroxide, dehydrated, and embedded in epoxylysine. Semithin sections (0.5-0.7 µm) were mounted on silanized glass slides, stained with toluidine blue, and observed with conventional light microscopy.

QUANTITATIVE ANALYSIS

Sensory retinal thickness (SRT) and outer retinal thickness (ORT) were measured in temporal retina at a distance 1 to 2 disc diameters from the temporal margin of the optic disc. The disc diameter was determined from the distance between the edges of the red reflective layer, which delineates the posterior boundary of the retina and terminates at the margin of the optic disc. This layer probably corresponds to the retinal pigment epithelium (RPE) and choriocapillaris (Figure 1).8 Another highly reflective red layer in pseudocolor scale delineating the surface of the retina in OCT image may correspond to retinal nerve fiber layer (NFL), including the internal limiting membrane (ILM).7 Measurement of SRT and ORT was performed manually using LRP in a scan profile program of OCT (Figure 2).16,17 For the SRT measurement, cursors were placed at the steepest portion of each rising slope produced at the ILM and RPE. The ORT was measured as the distance between the descending slope anterior to the RPE and the anterior border of the RPE. This relative low reflectivity layer anterior to the RPE may correspond to the photoreceptor layer.16 Retinal thickness of each eye represented the average of 10 measurements at different regions in the right eye of each mouse.

STATISTICAL ANALYSIS

The analyses were performed using the StatView (Abacus Concepts Inc, Berkeley, Calif) statistical analysis package. The SRT and ORT in each group were compared using the 1-way factorial analysis of variance and Fisher protected least significant difference for post hoc test. Values of P < .05 were considered significantly different. All data were expressed as mean ± SD.
responded with histologic findings. Previous reports in animal models compared normal and degenerative retina. Those studies defined the exact relationships between the cross-sectional image and the microanatomy of the retina; however, the potential to detect microanatomical change in degenerative retina using OCT is still unknown. Our findings indicated that the quantitative analysis using OCT scan profile has potential to detect progressive change in retinal degenerative diseases.

The murine rds allele is a semidominant null allele that causes abnormal development of photoreceptors, followed by their slow degeneration. The wild-type sequence at the rds locus encodes a photoreceptor disc membrane protein named peripherin/RDS. The rds mice have been studied because it is thought that they represent a model for some forms of hereditary retinal degeneration occurring in humans. In rds mice, the outer seg-

Figure 1. Optical coherence tomogram of a section of the optic disc (A) and its corresponding histologic section (B) in a healthy mouse. Logarithm of reflectivity is mapped to a pseudocolor scale. Retinal layers are labeled as follows: nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner segments of photoreceptors (IS), outer segments of photoreceptors (OS), retinal pigment epithelium (RPE), and choriocapillaris (CC). Calibration bar is 100 µm.

Figure 2. Representative longitudinal reflectivity profile. Measurement cursors were placed at the surface of nerve fiber layer and retinal pigment epithelium to measure sensory retinal thickness (SRT). Outer retinal thickness (CRT) was measured as the distance between the descending slope anterior to the RPE and the anterior border of the retinal pigment epithelium and that was assumed to be the length of photoreceptor cells.

Figure 3. Optical coherence tomographic scans and the corresponding histologic sections (A) and longitudinal reflectivity profiles (B) in each group of healthy and 2-, 6-, and 60-week-old retinal degeneration slow (rds) mice. As the retinal degeneration progresses, the sensory retinal thickness and the outer retinal thickness decrease. Calibration bar is 50 µm. Arrowheads indicate the steepest portion of each rising slope produced at the internal limiting membrane (upper arrowheads) and retinal pigment epithelium (lower arrowheads). Arrows indicate the descending slope, representing the inner surface of the photoreceptor layer.
ments of photoreceptor cells fail to develop, and a progressive loss of photoreceptor cells occurs throughout life. In the present study, the cross-sectional image demonstrated the change of outer retinal structure was consistent with histologic features.

Several techniques have reportedly measured retinal thickness. Optical coherence tomography has original software for automatically measuring SRT, and it has been widely used in clinical study. Baumann et al have described the technique using a manually assisted method of computer software. In their study, measurement cursors were placed at the steepest portion of each rising slope produced at the ILM and RPE when the observer visualized the representative LRP. In our study, this technique was used to measure SRT, and SRT was measured clearly because the reflectivity from ILM and RPE could be obtained as a sharply rising slope. In contrast, the technique to measure ORT has not been established, and the other cursor was placed at the steepest portion of the descending slope of the outer nuclear layer to measure ORT in this study. Although this technique of measuring ORT should be evaluated in detail, our data showing the reduction of the outer retina in rds mice indicated that this technique may be effective in measuring ORT.

In summary, our findings suggested a possible relationship between SRT and ORT and histologic change in retinal degenerative diseases. Although the quantitative analysis obtained with OCT scans should be followed by functional tests, OCT has the potential to detect microanatomical change of degenerative retina and may be useful for noninvasive assessment of human retinal degeneration.

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