Transsynaptic Retinal Degeneration in Optic Neuropathies: Optical Coherence Tomography Study

Prema Sriram, Stuart L. Grabam, Chenyu Wang, Con Yiannikas, Raymond Garrick, and Alexander Klistorner

PURPOSE. Recently demonstrated neuronal loss in the inner nuclear layer of the retina in multiple sclerosis (MS) and glaucoma raises the question of a primary (possibly immune-mediated) or secondary (transsynaptic) mechanism of retinal damage in these diseases. In the present study we used optical coherence tomography to investigate retrograde transsynaptic degeneration in patients with long-standing and severe loss of ganglion cells due to optic neuropathy.

METHODS. Fifteen eyes of glaucoma patients with visual field defect limited to upper hemifield and 15 eyes of MS patients with previous episode of optic neuritis (ON) and extensive loss of ganglion cells were imaged using spectral-domain optical coherence tomography and compared with two groups of age-matched controls. Combined retinal ganglion cell layer/inner plexiform layer (GCL/IPL) thickness and inner nuclear layer (INL) thickness were analyzed.

RESULTS. In the glaucoma group there was a significant (P = 0.0005) reduction of GCL/IPL thickness in the lower (affected) retina compared with normal controls; however INL thickness was not statistically reduced (P = 0.49). In the MS group reduction of GCL/IPL thickness in both hemifields of ON eyes was also significant (P = 0.0001 and P < 0.0001 for inferior and superior retina respectively). However, similar to the glaucomatous eyes, there was no significant reduction of INL thickness in both hemifields (P = 0.25 and P = 0.45).

CONCLUSIONS. This study demonstrates no significant loss of INL thickness in parts of the retina with long-standing and severe loss of retinal ganglion cells. (Invest Ophthalmol Vis Sci. 2012; 53:1271–1275) DOI:10.1167/iovs.11-8732

Transsynaptic degeneration has recently attracted considerable interest as one of the possible mechanisms of neurodegeneration in multiple sclerosis (MS) and glaucoma. The visual system represents an ideal model to study transsynaptic degeneration because it comprises relatively independent pathways of hierarchically linked neurons and can be studied not only morphologically, but in vivo. The process of transsynaptic degeneration has been documented in the visual system from the ganglion cell layer (GCL) extending proximally but this still remains unproven within the retina. Neuronal loss in layers of the retina outside of the GCL has been demonstrated pathologically and electrophysiologically in human glaucoma and multiple sclerosis, and raises the question of whether the mechanism for this cell loss is a primary degeneration (possibly immune-mediated) or secondary on the basis of transsynaptic degeneration.

An experimental way to study retinal transneuronal degeneration is using optic nerve axotomy. While few animal studies failed to find pathologic changes distal to GCL after optic nerve axotomy, a reduction of the inner nuclear layer after long survival times has been demonstrated by Holland et al. Optic neuropathies such as glaucoma and optic neuritis damage axons of retinal ganglion cells, which causes retrograde axonal degeneration and finally results in neuronal death. One should not, however, expect to find changes in inner nuclear layer compared with healthy individuals unless it is caused by retinal transsynaptic degeneration.

Optical coherence tomography (OCT) permits segmentation and measurement of retinal layers in vivo by using selective reflectivity of backscattered near-infrared light by different retinal layers. The spectral-domain OCT technique demonstrates axial resolution in the range of few microns and allows clear visualization of individual retinal layers, allowing evaluation of retinal transsynaptic degeneration in vivo.

The aim of the present study, therefore, was to employ such high-resolution spectral-domain OCT imaging to identify evidence of possible inner nuclear layer (INL) degeneration in post optic neuritis MS and primary open angle glaucoma (POAG) patients with long-standing severe loss of retinal ganglion cell layer (RGL).

MATERIALS AND METHODS

Two groups of patients were enrolled in the study. The first group comprised of 15 POAG patients with dense visual field defects ( Humphrey 10-2 program) limited to superior hemifield. All patients had at least 20 points in the superior hemifield with P < 0.005 and not >2 non-rim points with P < 0.02 in the inferior hemifield on pattern deviation plot. The diagnosis of glaucoma was based on the finding of a typical excavation of the optic disc with neural rim loss corresponding with visual field loss in that hemifield. The visual fields were reproducible (same defect >3 fields) and reliable (fixation losses, false negatives, and false positives all <30%). Raised intracocular pressure was not a criterion for diagnosis.

The second group was comprised of 15 patients who had clinically definite multiple sclerosis (CDMS) and a unilateral episode of optic neuritis (ON) at least 3 years before the study. Average time since onset of ON was 3.7 ± 0.8 years (range 3 to 6 years). The diagnosis of CDMS was based on the criteria of McDonald et al. Optic neuritis was diagnosed by a neuro-ophthalmologist based on clinical findings. Exclusion criteria were an atypical presentation, recurrent ON, and a history of other ocular or neurologic disease.
Thirty-two age- and sex-matched controls were examined with the OCT, 17 for the MS group and 15 for the POAG group. The eligibility criteria for control subjects included 6/6 vision in both eyes and normal ophthalmic examination. All normal subjects underwent complete ophthalmic examination and were found normal on the slit lamp biomicroscope, and dilated fundus examination. They also had normal visual fields on the Humphrey 24-2 SITA (Swedish interactive thresholding algorithm) standard program. One eye of each normal subject was selected randomly.

The study was approved by the Institutional review board. Procedures followed the tenets of the Declaration of Helsinki and informed consent was obtained from all participants.

OCT Recording and Analysis
Optical coherence tomography was performed (Spectralis HRA + OCT; Heidelberg Engineering, Heidelberg, Germany). A radial protocol using a star-like pattern of line scans centered on the macula with resolution of 1536 pixels was used (Fig. 1). The criteria for a good quality scan included signal strength greater than 25, good centration of the scan, and uniform brightness. Scans that satisfied these criteria were taken for analysis. Analysis was performed on vertical scan only. One hundred scans were averaged for each line scan. Thirty degrees of visual angle (15° of eccentricity) were scanned, but only the central 14° (7° of eccentricity) were used for analysis, because the definition of layers becomes much less distinct beyond that. Retinal layers were segmented automatically using a custom designed algorithm which applied vessel detection and removal, multiple size median filtering, and Canny edge detection to identify borders of retinal layers.

The GCL and inner plexiform layer (IPL) were combined together (GCL/IPL) because the border between them was indistinguishable in several subjects, while the inner nuclear layer (INL) was analyzed on its own (see example in Fig. 1). The thickness of each layer was measured at seven points for each hemifield, which were equally distributed between 1.5° and 7° of eccentricity.

Statistics
Statistical analysis was performed using statistical software (SPSS 11.0 for Windows; SPSS, Chicago, IL). Mean values of GCL/IPL and INL thickness were compared between an aged-matched control, affected and nonaffected hemifields of glaucomatous eye, or affected and fellow eyes (hemifield-based) of MS patients using Student’s t-test. Significance was assessed at the $P < 0.05$ level.

RESULTS
All subjects achieved good quality scans (dB > 20). The demographic data for each group are presented in Table 1.

Glaucoma Group
Averaged Humphrey mean deviation (MD) of affected (superior) hemifield was $-23.7 \pm 4.9 \text{ dB}$ and of nonaffected (lower) hemifield $-1.75 \text{ dB}$ ($P < 0.0001$). There was a significant (29.0%, $P = 0.00037$, paired t-test) reduction of GCL/IPL thickness in the lower retina compared with normal controls (Table 2). Reduction was significant at all eccentricities studied for the affected hemifield ($P < 0.02$ for all points) (Fig. 2A). Figure 5 represents an example of Humphrey visual field and OCT scan of an affected eye of one of the glaucoma patients.

While thickness of GCL/IPL in the upper hemifield was also significantly reduced ($P = 0.005$), reduction was of considerably smaller magnitude (11.7%) and was nonsignificant for first, second, third, and seventh points (Table 2). There was no difference for any of the points tested ($P > 0.2$, for all points) (Fig. 2B). INL thickness in nonaffected hemifield (upper retina) was similarly not reduced for both averaged ($P = 0.94$), as well as individual points ($P > 0.1$ for all points) (Table 2).

Multiple Sclerosis Group
Because optic neuritis predominantly affects central fibers of the optic nerve without upper or lower field preponderance, both hemifields of the affected eye were considered abnormal. There was a significant reduction of GCL/IPL thickness in both hemifields of ON eye (28.5% and 30.5% reduction, $P = 0.0001$ and $P < 0.0001$ for inferior and superior retina, respectively) (Table 2). Reduction was significant at all eccentricities for both affected hemifields ($P < 0.02$ for all points) (Fig. 4A).

Non-ON eyes also demonstrated a significant reduction of GCL/IPL thickness in both hemifields, albeit on a lesser scale (9.7% and 10.6% reduction, $P = 0.0003$ and $P < 0.0001$ for inferior and superior retinas respectively). The thickness of INL, however, did not differ significantly from the normal controls in both hemifields of ON eyes ($P = 0.1$ and $P = 0.12$ for inferior and superior retina respectively) (Fig. 4B). Non-ON eyes also had normal INL thickness ($P = 0.1$ and $P = 0.12$ for inferior and superior retina respectively).

DISCUSSION
The presence of transsynaptic degeneration in the visual system is well documented. There is strong evidence of anterograde and retrograde degeneration between retinal ganglion cells (RGCs) and target neurons in higher visual areas. Anterograde degeneration of lateral geniculate nucleus (LGN) cells, optic radiation, and cortical neurons after loss of RGC was demonstrated in human and experimental glaucoma and in multiple sclerosis. Similarly, retrograde transsynap-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Demographic Data for Each Group} & \textbf{Glucoma} & \textbf{Glucoma control} & \textbf{MS} & \textbf{MS control} \\
\hline
\textbf{Age (y)} & 64.1 $\pm$ 9.8 & 63.8 $\pm$ 10.3 & 37.1 $\pm$ 8.5 & 36.4 $\pm$ 9.6 \\
\textbf{Male/Female} & 7/8 & 6/9 & 6/9 & 7/10 \\
\textbf{Visual Acuity} & & & & \\
\hline
\end{tabular}
\caption{Demographic Data for Each Group}
\end{table}
tic changes in RGC after cortical lesions were found in animals and human studies.\textsuperscript{21,22} Expansion of transsynaptic changes into the retina, however, remains controversial. Recently a deficit of bipolar cells in the retina of MS patients (identified pathophysiologically) has been suggested as possible evidence of transsynaptic retinal degeneration.\textsuperscript{1} However, a similar abnormality in outer nuclear layer reported in the same study makes this explanation less likely.\textsuperscript{8}

An OCT study of the retina in MS patients recently published by Saidha et al.\textsuperscript{14} demonstrated that reduction of retinal nerve fiber layer (RNFL) and GCL/IPL thickness after an episode of ON was not accompanied by INL thinning. However, the follow-up period was probably not long enough to verify the existence of transsynaptic degeneration because patients as early as 3 months after acute ON may have been included. This study, on the other hand, demonstrated that there is a subgroup of MS patients (approximately 10%) in whom the thickness of all retinal layers is reduced with RGC layer producing largest degree of reduction (16%) compared with INL (6.6%) and ONL (6.7%). Whether this is a result of transsynaptic (anterograde or retrograde) retinal degeneration or a consequence of primary retinal pathology in MS or simply an artificially selected group representing the low end of the spectrum of the retinal thickness\textsuperscript{23} is not clear.

Similar results were demonstrated by Wang et al.\textsuperscript{24} in glaucoma patients. Authors concluded that RNFL and GCL/IPL thickness is reduced while INL thickness remains unchanged. However, spectrum of the visual field loss in the study patients varied widely and the topographic relationship between visual field sensitivity and GCL/IPL loss was examined only in one patient.

Therefore, in the present study we used clear-cut cases of optic neuropathies with substantial and long-standing RGC loss. We specifically selected glaucoma patients with severe perimetrical field loss confined to one (upper) hemifield which indicates extensive, but relatively localized loss of RGC. While normal result of standard perimetry in lower hemifield does not preclude loss of ganglion cells (and, in fact, we did find moderate reduction of GCL/IPL thickness in perimetrical normal upper retina), it is not likely to exceed 20%.\textsuperscript{25}

Similarly, in the MS group we selected subjects with severe unilateral post-ON loss of RGC (of similar magnitude to glau-

### Table 2. Hemifield GCL/IPL and INL Thickness in Glaucoma and ON Patients and Normal Controls

<table>
<thead>
<tr>
<th></th>
<th>GCL/IPL Thickness ($\mu$)</th>
<th>INL Thickness ($\mu$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glaucanma Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glaucanmatous eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected (upper) hemifield</td>
<td>52.4 ± 12.0$^*$</td>
<td>41.9 ± 7.3</td>
</tr>
<tr>
<td>Fellow (lower) hemifield</td>
<td>66.6 ± 14.9$^*$</td>
<td>41.5 ± 5.3</td>
</tr>
<tr>
<td>Age-matched normal controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper hemifield</td>
<td>73.7 ± 13.4</td>
<td>42.1 ± 5.1</td>
</tr>
<tr>
<td>Lower hemifield</td>
<td>75.4 ± 12.1</td>
<td>41.3 ± 4.1</td>
</tr>
<tr>
<td><strong>MS Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ON eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper hemifield</td>
<td>57.4 ± 7.6$^*$</td>
<td>43.8 ± 4.5</td>
</tr>
<tr>
<td>Lower hemifield</td>
<td>60.0 ± 7.2$^*$</td>
<td>43.2 ± 4.1</td>
</tr>
<tr>
<td>Fellow eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper hemifield</td>
<td>71.4 ± 11.2$^*$</td>
<td>44.0 ± 5.2</td>
</tr>
<tr>
<td>Lower hemifield</td>
<td>73.8 ± 11.5$^*$</td>
<td>42.4 ± 4.7</td>
</tr>
<tr>
<td>Age-matched normal controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper hemifield</td>
<td>80.2 ± 13.5</td>
<td>42.4 ± 5.2</td>
</tr>
<tr>
<td>Lower hemifield</td>
<td>83.4 ± 11.4</td>
<td>43.0 ± 4.1</td>
</tr>
</tbody>
</table>

* Statistically significant difference between patients and normal controls.

### Figure 2. Averaged thickness of GCL/IPL (A) and INL (B) in affected (left) and nonaffected (right) hemifield of glaucoma patients (black columns) and corresponding hemifield of age-matched controls (gray columns).
comatous eyes), which was a result of inflammatory transection of optic nerve fibers and subsequent retrograde degeneration. While there was significant reduction of GCL in fellow eyes, the degree of thinning was modest compared with the ON eye. The thinning of RNFL in fellow eyes is most likely a result of subclinical inflammation in a visual pathway of the fellow eye, which is not uncommon in MS.

In our subjects the loss of RGC was relatively long-standing because glaucoma is a slowly-progressing disease, while all MS patients had an episode of ON at least 3 years before the study. Despite the severity of RGC loss and its duration we did not find evidence of INL thinning in either of the groups. While the most likely explanation of the result observed is an absence of transsynaptic degeneration in studied conditions, other possibilities must be considered. The INL includes not only nuclei of bipolar cells, but also horizontal cells, amacrine cells, interplexiform cells, and supportive Müller cells. Even though the percentage of bipolar cells in INL is by far the largest, the presence of other cell types may obscure detection of bipolar cell decline. Transsynaptic degeneration can be an extremely slow process, which may take many years to manifest. For example, the duration of disease in a postmortem study, which demonstrated INL abnormalities in MS patients was >20 years. A recent study, however, suggested that retrograde transsynaptic degeneration in human optic tract is functionally apparent as early as 18 months after cortical damage.

**FIGURE 3.** A typical example of Humphrey visual field (upper row) and retinal thickness profile (lower row) of a glaucoma patient. Note absence of RNFL and significant thinning of GCL/IPL in the upper retina (left side).

**FIGURE 4.** Averaged thickness of GCL/IPL (A) and INL (B) in inferior (left) and superior (right) hemifield of MS patients (black columns) and corresponding hemifield of age-matched controls (gray columns).
tionally, a long delay of structurally visible changes compared with more subtle functional alteration is another possibility. In fact, functional impediment of INL has been reported in both conditions.7,31–33 Finally, changes in cellular composition of the overlying retinal layers (extensive loss of RNFL and ganglion cells) can potentially alter light reflectivity of INL structures and therefore influence the measurement of INL thickness. Our study is also limited by relatively small sample size and cross-sectional study design.

In summary, our study demonstrated no significant loss of INL thickness in parts of the retina with long-standing and severe loss of RGC in patients with optic neuropathies.

References


