Elevated Intraocular Pressure Induces Amyloid-β Deposition and Tauopathy in the Lateral Geniculate Nucleus in a Monkey Model of Glaucoma

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PURPOSE. Recent evidence has suggested a potential association between Alzheimer’s disease (AD) and glaucoma and found significant deposition of amyloid-β (Aβ) and Tau protein in the retinas of glaucoma patients. However, no coherent finding has emerged regarding the Aβ-like changes in the central visual system (CVS). Studies confirming the presence of Aβ and Tau neuropathology are warranted to identify the underlying mechanism that contributes to the visual impairment observed in glaucoma.

METHODS. A chronic glaucoma model was established in rhesus monkeys. The retina, optic nerve, CVS including the lateral geniculate nucleus (LGN) and primary visual cortex (V1), and cognitive areas including the hippocampus (Hpp) were evaluated. Aβ 1-42 and phosphorylated-Tau (p-Tau) were tested in the aforementioned structure using immunohistochemistry, Western blotting and ELISA, and the neuritic plaques and argyrophilic structures/neurofilaments were observed using silver staining and transmission electron microscopy (TEM).

RESULTS. Immunohistochemistry revealed positive Aβ and p-Tau labeling in the LGN. According to Western blotting assay and ELISA, Aβ and p-Tau were present in the LGN. Aβ also was expressed weakly in the primary visual cortex. In contrast, the hippocampus, which is the most severely affected region in AD, showed no positive labeling. Structurally, silver staining and TEM revealed neuritic plaques and argyrophilic structures/neurofibrillary tangles, in the LGN.

CONCLUSIONS. For the first time to our knowledge, these data collectively establish the existence of hallmark AD-like pathologies in the glaucomatous LGN. Our results may provide new targets for developing research therapies that will enhance neuroprotection in glaucoma patients.

Keywords: glaucoma, lateral geniculate nuclei, visual cortex, Alzheimer’s disease
Surprisingly, no coherent picture has emerged regarding the changes of Aβ and Tau protein that occur in the central visual system (CVS) following injury to RGCs. Studies confirming the presence of Aβ and Tau neuropathology are needed to identify the underlying mechanisms that contribute to the visual impairments observed in glaucoma. Thus, we sought to achieve this objective using our glaucomatous monkey model.

**Materials and Methods**

**Ethics Statement**

This study strictly adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and was approved and monitored by the Institutional Animal Care and Use Committee of Zhongshan Ophthalmic Center (Permit Number: SYXK (YUE) 2010-0058).

**Subjects and Procedures**

A total of 12 healthy adult rhesus monkeys (both sexes; Blue Island Biological Technology Co., Ltd., Guangdong, Guangzhou, China, Qualification), with initial body weights of 7 to 12 kg and initial ages of 4 to 6 years, were used in this study. Because a monocular glaucoma model does not sufficiently imitate the natural course of glaucoma or the pathogenic process of CVS injury in patients, a binocular glaucoma model was established in this study. Various measures were used to minimize the animals' discomfort. Animal health was monitored daily by animal care staff and veterinary personnel.

Bilateral chronic IOP elevation was induced in six rhesus monkeys by laser photocoagulation (VISUALS Trion & LSL Trion Laser Slit Lamp; Carl Zeiss Meditec AG, Jena, Germany) of the trabecular meshwork in both eyes as reported previously, and another six normal monkeys that did not receive laser photocoagulation were used as controls. IOP was monitored weekly between 9:00 AM and 12:00 PM with a Tono-Pen XL tonometer (Reichert, Depew, NY, USA) before and after laser treatment. If the IOP was not consistently higher than 24 mm Hg, additional laser treatments were performed daily by animal care staff and veterinary personnel.

For Aβ and Tau IHC study, the sections were incubated overnight at 4°C in different primary antibodies (Aβ 1-42, rabbit polyclonal, 1:100; AB5078P; Millipore, Billerica, MA, USA; p-Tau, AT8 clone, mouse monoclonal, 1:200, MN1020; Thermo Fisher Scientific, Rockford, IL, USA), processed, and visualized with horseradish peroxidase and dianaminobenzidine according to the protocol provided by the kit (Vector Laboratories, Burlingame, CA, USA). To measure semiquantitatively the expression of p-Tau in neurons, p-Tau-positive cells were counted in bilateral cerebral sections of each monkey and the average value of the above two were calculated. For neuron-specific nuclear protein (NeuN) and p-Tau double labeling, the sections were labeled with NeuN (rabbit polyclonal, 1:200, ab104225; Abcam, Cambridge, MA, USA) and p-Tau (AT8 clone, mouse monoclonal, 1:50, MN1020; Thermo Fisher Scientific) overnight at 4°C, and antibody bindings were visualized by incubating the sections with donkey anti-rabbit Alexa Fluor 555 and donkey anti-mouse IgG Alexa Fluor 488 secondary antibodies (1:500, Invitrogen, Carlsbad, CA, USA) and p-Tau (AT8 clone, mouse monoclonal, 1:50, MN1020; Thermo Fisher Scientific) overnight at 4°C, and antibody bindings were visualized by incubating the sections with donkey anti-rabbit Alexa Fluor 555 and donkey anti-mouse IgG Alexa Fluor 488 secondary antibodies (1:500, Invitrogen, Carlsbad, CA, USA), respectively. The cell nuclei were stained with DAPI (1:500, Sigma-Aldrich Corp.). Images were obtained using a Zeiss LSM 510 META confocal laser-scanning microscope (Carl Zeiss Meditec).

For silver staining, sections were stained with a modified Bielschowsky stain using Hito Bielschowsky’s OptiStainTM Kit (HitoBiotech, Inc., Claymont, DE, USA). All images were processed with Adobe Photoshop CS6 software (Adobe Systems, Seattle, WA, USA).

**Histology and Immunohistochemistry (IHC)**

The removed optic nerves and brains tissues were fixed in 4% paraformaldehyde for 24 to 48 hours, and then the brains were dehydrated in graded ethanol solutions, biopsied along the coronal plane, and embedded in paraffin. The sections were approximately 5 μm thick.

For Nissl staining, the optic nerve specimens described above were rinsed in cacodylate buffer, postfixed in 2% osmium tetroxide, dehydrated in an ascending series of alcohol, and embedded in epoxy resin. Semithin cross-sections (1 μm) of the optic nerves were cut and stained with 0.5% toluidine blue (Sigma-Aldrich Corp., St. Louis, MO, USA) to evaluate optic nerve damage. The diameters of the optic nerve were compared between the two groups. The LGN sections also were stained with 0.5% toluidine blue to evaluate senior visual neuronal atrophy. The images were obtained using AxioVision Release 4.8 software from a spinning disk inverted microscope (Axioplan-2 Imaging; Carl Zeiss Meditec).

For Aβ and Tau IHC study, the sections were incubated overnight at 4°C in different primary antibodies (Aβ 1-42, rabbit polyclonal, 1:100; AB5078P; Millipore, Billerica, MA, USA; p-Tau, AT8 clone, mouse monoclonal, 1:200, MN1020; Thermo Fisher Scientific, Rockford, IL, USA), processed, and visualized with horseradish peroxidase and dianaminobenzidine according to the protocol provided by the kit (Vector Laboratories, Burlingame, CA, USA).

**Tissue Processing**

After we made sure that the elevated IOP was maintained for more than 6 months, the RNFLs in the model group were decreased more than 50% and the optic cup-to-disc ratios exceeded 0.8, the six model animals and six control animals were killed. Among them, three model and three control animals were deeply anesthetized and subjected to cardiac perfusion with 1500 mL 0.01 M PBS and 2500 mL of a mixture of 4% paraformaldehyde and 1% glutaraldehyde, after which the optic nerve (1–3 mm behind the globe) and brain were quickly dissected, followed by removal of the lateral geniculate nuclei (LGN), primary visual cortex (V1, which receives sensory inputs from the LGN), and the hippocampus (Hpp). These brain tissues were prepared for immunohistochemical and histologic analysis according to different protocols listed below. The other three model and three control animals also were deeply anesthetized, but in these animals, we performed cardiac perfusion with only 1500 mL 0.01 M PBS. Then, the same tissues listed above were dissected and kept in a −80°C freezer. The optic nerve was fixed using the same solutions described above. These brain tissues were prepared for Western blot and ELISA analysis according to different protocols listed below.

**Western Blot Analysis**

The snap-frozen LGN, V1, and Hpp sections were thawed and the total proteins were extracted and processed for Western
Enzyme-Linked Immunosorbent Assay

The levels of Aβ 1-42 and p-Tau in the snap-frozen LGN, V1, and Hpp tissues were measured. The samples were homogenized in guanidine-Tris buffer (5.0 M guanidine HCl containing 50 mM Tris-HCl, pH 8.0), and the homogenates were incubated at room temperature for 4 hours before they were assayed. The levels of Aβ 1-42 and p-Tau were quantified using commercial ELISA kits (Aβ 1-42; Signet Laboratories, Dedham, MA, USA; p-Tau, Invitrogen, Grand Island, NY, USA) following the manufacturers’ protocols. The absorbance of the plates was read at 450 nm with a microplate reader (NB-EXL-80; Bio-Tek, Winooski, VT, USA). The standard curves were established using a variety of concentrations of standard Aβ 1-42 and standard p-Tau (15.6-1000 pg/mL). The data are expressed as nanograms for Aβ 1-42 or p-Tau per milligram of brain tissues.

Statistical Methods

The IOPs from prelaser-treated and control eyes were compared using independent t-tests. The data from pre- and postlaser-treated eyes, including IOP and RNFL thickness, were compared using paired t-tests. Quantitative comparisons of the diameters of the optic nerve, numbers of p-Tau-positive cells, ELISA-determined Aβ 1-42 and p-Tau levels in different sections were performed between the glaucomatous and control groups using independent t-tests. All assays were performed at least in triplicate. All statistical assessments were two-sided, and P < 0.05 was considered significant. The data are presented as the means ± SD. All analyses were performed using SPSS 13.0 software (Chicago, IL, USA).

RESULTS

Glucomatous Characteristic Changes After Laser Photocoagulation

Intraocular Pressure. The IOPs of control and model groups before photocoagulation treatment were 16.9 ± 1.9 and 17.5 ± 2.6 mm Hg, respectively, a difference that was not statistically significant (P > 0.05). The IOP of the model group began to increase slowly 0.5 to 3.5 weeks after photocoagulation. The elevated IOP was sustained for 6 to 12 months at an average IOP of 35.7 ± 7.6 mm Hg, which was significantly different (P < 0.05) than the IOP of the prelaser-treated and control animals.

RNFL and the Optic Disc Cupping. The average RNFL thicknesses in the model group decreased to different degrees (mean = 45 ± 11 μm) following IOP elevation (Fig. 1A1), which was significantly different (P < 0.05) compared to the prelaser-treated (94 ± 13 μm) and control (97 ± 8 μm) groups (Fig. 1A2). Following IOP elevation, the optic disc cupping also expanded simultaneously to different extents (Fig. 1B1).

Atrophy in Optic Nerve and LGN. The overall appearance of the optic nerve and LGN in the model group appeared...
to be atrophic compared to the controls (Figs. 1C1, 1D1). The diameter of the optic nerve in the model group (1.2 ± 0.1 mm) was smaller than that of the control group (2.0 ± 0.1 mm, \( P < 0.05 \), Fig. 1E). The LGN also shrank and the cellular layers became unclear and even more indistinct (Fig. 1D1). By contrast, in the controls, the diameter and structure of the optic nerve were normal (Fig. 1C2), the size of the LGN was normal, and the cellular layers were clearly distinct (Fig. 1D2).

**Histologic Characterization**

**Immunohistochemistry.** Positive A\(\beta\) 1-42 cells were observed in glaucomatous LGN (Fig. 2A), whereas no A\(\beta\) 1-42 labeling was found in the control LGN or the other sections of both groups (Figs. 2B-F). Positive p-Tau cells were observed in all sections of both groups (Figs. 2G-L), especially in glaucomatous LGN (Fig. 2G). Tau-positive cells per 1 mm\(^2\) area in glaucomatous LGN were significantly more frequent than those in the control group (\( P < 0.05 \)); however, there were no significant differences between the two groups in V1 and Hpp (\( P > 0.05 \), Fig. 2M). Double immunostaining for Tau and NeuN in the LGN revealed that the positive p-Tau was located in the neuronic cytoplasm (Fig. 3).

**Silver Staining.** Silver-based staining has been used to identify pathologic changes in neurons containing altered A\(\beta\) and Tau proteins in AD. In the glaucoma model group, the positive labelings, that is, neuritic plaques (Fig. 4A) and argyrophilic structures (Fig. 4B), were present in LGN when the modified Bielschowsky silver stain was used. This staining was absent in the control LGN (Fig. 4C), model V1 (Fig. 4D), and Hpp of both groups (Hpp data not shown).

**Transmission Electron Microscopy.** Ultrastructurally, the malnourished neuronal axons twined into clustered neuritic plaques, which gathered outside of the neurons, and swollen myelin was observed in glaucomatous LGN (Fig. 5A). NFTs were present in the neuronal cytoplasm (Fig. 5B). However, these pathologic changes were absent in all brain tissues in the control LGN (Fig. 5C), model V1 (Fig. 5D), and Hpp of both groups (Hpp data not shown).

**Western Blot**

Western blot analysis showed that A\(\beta\) 1-42 and p-Tau bands were expressed positively in the LGN and that A\(\beta\) 1-42 also was expressed weakly in V1 of the glaucomatous model animals. However, the other samples showed negative expression in the model and control groups (Figs. 6A, 6B).

**Enzyme-Linked Immunosorbtent Assay**

To quantitatively compare the A\(\beta\) 1-42 and p-Tau levels in different sections between the glaucomatous and control groups, ELISA analysis was performed using the brain homogenates. The glaucomatous LGN and V1 sections exhibited significantly increased A\(\beta\) 1-42 levels (mean concentrations of 22.2 and 2.9 ng/mg tissue, respectively) compared to the controls (mean concentrations of 1.1 and 1.0 ng/mg tissue, respectively, Fig. 6C). In the glaucomatous LGN, p-Tau expression also was robust, with a mean concentration of 63.0 ng/mg tissue; by contrast, the control LGN displayed a mean concentration of 4.1 ng/mg tissue (Fig. 6D). There were no significant differences between the glaucomatous and control groups in either the A\(\beta\) 1-42 levels in the Hpp or the p-Tau levels in the V1 or Hpp.

**DISCUSSION**

We used laser to establish a chronic glaucoma model in rhesus monkeys. We then conducted a pathologic examination of the CVS following injury to RGCs. Using an approach that combined IHC, Western blotting, ELISA, silver staining, and TEM techniques, we found that hallmark AD-like pathologies, such as A\(\beta\) 1-42 and p-Tau deposition, were present in the glaucomatous LGN.

The anatomic structure of the CVS and the pathologic conditions in the retina and optic nerve in simian ocular hypertension models are surprisingly similar to those observed in human patients.\(^{16,17}\) Therefore, in our study, an experimental chronic glaucoma model was induced successfully in rhesus monkeys via laser photocoagulation. The previous studies that used the monocular model mostly focused on the glaucomatous retinal ganglion cells and optic nerve, so the contralateral eye could be used as a control. However, our research focused on visual centers, which receive input from both eyes. Therefore, it is very difficult to process the level of cellular damage in the LGN of the monocular model due to the reduced pathology resulting from only a single affected eye. This is true particularly for TEM studies, which are essential to identify neuritic plaques in AD. Thus, we chose a bilateral glaucoma model to avoid the false-negative results. In addition, the bilateral glaucoma model has been reported previously.\(^{18,19}\) In addition, because the Hpp is one of the first and leading brain structures to develop pathologic changes in AD patients,\(^{20}\) the Hpp also was studied in the model group.

There is emerging evidence suggesting that glaucoma also affects other components of the visual pathway. Glaucomatous neuronal death occurs in the retina, optic nerve, LGN, and the visual cortex. Neuropathologic examination revealed marked degenerative changes, including neuron shrinkage and loss\(^{21,22}\) in the LGN, which was accompanied by reactive astrogliosis\(^{23}\) or glial activation.\(^{24}\) Magnetic resonance (MR) techniques are well suited for evaluating the brain changes in vivo. In turn, studies have demonstrated decreases in LGN volume\(^{25}\) and visual cortex thickness.\(^{26}\) Functional MR showed decreased response in the visual cortex after stimulation of the glaucomatous eye.\(^{27}\) Therefore, these mechanisms are similar to those first described in neurodegenerative diseases, which comprise a heterogeneous group of disorders with clinical and pathologic diversity, including AD, Parkinson’s disease, and amyotrophic lateral sclerosis. In agreement with previously published data,\(^{21,22,25,28}\) in the present glaucomatous monkey model, the average RNFL thicknesses decreased, the optic nerve cupping expanded, and the optic nerve and LGN appeared atrophic. The LGN also shrank apparently and its cellular layers became unclear and even more indistinct. It appears that the loss of significant portions of the visual field in human glaucoma may be associated with pathologies in the visual centers of the brain that are similar to the features observed in neurodegenerative diseases.

Numerous similarities exist between glaucoma and AD. Both are slow, chronic neurodegenerative disorders with age-related incidences. AD is the most common type of dementia and is characterized by extracellular deposits of A\(\beta\) in the form of parenchymal plaques and cerebral amyloid angiopathy coexisting with intraneuronal accumulations of p-Tau in the form of NFTs.\(^{29}\) Previous in vivo and in vitro studies have demonstrated that glaucoma may lead to hallmark neuropathies in the retina that are similar to those described in AD. At a molecular level, caspase activation induces abnormal amyloid precursor protein (APP) formation, which is the key event in the pathogenesis of AD, and has been observed in a rat model of chronic ocular hypertension.\(^{30}\) Very recent data have shown...
FIGURE 2. Aβ 1-42 deposition is present outside of the cells in the glaucomatous LGN, and p-Tau is expressed apparently in the cytoplasm of glaucomatous LGN. Representative IHC images of Aβ 1-42 (A–F) and p-Tau (G–L). Arrows indicate positive Aβ 1-42 deposition (A) or positive p-Tau cells (G–L). (G, Glaucoma; C, Control). (M) Quantitation of positive p-Tau cells per 1 mm² area (G, n = 6; C, n = 6). *P < 0.05. Error bars: means ± SD.
that Aβ is increased in the optic nerve and RGC layer in experimental glaucoma models.9,10,31 Tau immunoreactivity also has been observed in the glaucomatous retina.12 For the first time to our knowledge, our study has demonstrated the presence of AD-like pathology in glaucomatous CVS. Silver staining can be performed easily to demonstrate plaques and NFTs on paraffin sections.32 The features identified by silver staining were quite similar to the characteristics observed using anti-Aβ and anti-Tau immunostaining. In addition, the TEM used in this study provided useful information for examining the ultrastructural aspects of amyloid plaques and NFTs.33,34

Aβ, which normally is found as a soluble monomeric component in biological fluids and brain interstitial fluid, is the main fibrillar constituent of brain deposits. Increasing data indicate that neither the soluble forms nor the deposited fibrils exert neurotoxicity. However, intermediate conformations, which typically are composed of low molecular mass oligomers and short protofibrils less than 200 μm in length, mostly Aβ 1-42, now are considered to be the likely neurotoxic species that trigger cell death.35 Tau proteins are important for the stabilization and assembly of microtubules, and in turn, they affect the intraneuronal transport of cargos. Thus, under aberrant conditions, the dysregulation of Tau proteins may lead

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**Figure 3.** P-Tau are coexpressed with NeuN in neurons and expressed apparently in the model LGN of group than that of the control. Representative double immunostaining images of P-Tau and NeuN in the model (A) and control (B) groups. Arrows indicate positive coexpressing of p-Tau and NeuN. Error bars: 5 μm.

**Figure 4.** Bielschowsky silver staining reveals neuritic plaques and argyrophilic structures in the model group. Bielschowsky silver staining reveals neuritic plaques (A, arrows) and argyrophilic structures (B, arrows), two hallmark features of AD in glaucomatous LGN. However, these abnormal structures are absent in the control LGN (C) and in the model V1 (D).
to dysfunctional axonal transport. The phosphorylation state of Tau alters its intrinsic functions and binding affinity to microtubules. P-Tau proteins aggregate into oligomers and fibrils, and then form NFTs consisting of paired helical filaments in the somatodendritic compartments of neurons.

These observations raise the intriguing possibility that underlying AD-like pathology contributes to the visual impairment observed in glaucoma. Studying these hallmark pathologies may result in a paradigm shift in the management of ocular diseases. It has been shown that targeting different components of the Aβ formation and aggregation pathway can reduce glaucomatous RGCs apoptosis in vivo and, therefore, raises the possibility of using neuroprotective mechanisms to combat glaucoma.

In our study, the presence of Aβ 1-42 and abnormal p-Tau, as determined by Western blot analysis and ELISA, was somewhat different. Apart from the fact that both were found in the glaucomatous LGN, Aβ 1-42 also was expressed weakly in the V1 of glaucoma model animals. In accordance with previous studies, we considered it likely that these AD-like pathologic observations may indicate disease progression that Aβ deposition may occur before tauopathy. According to the Aβ hypothesis, AD begins with the abnormal processing of the transmembrane APP. The proteolysis of extracellular domains by sequential β and γ secretases results in a family of peptides that form predominantly β-sheets, the Aβ. The more insoluble of these peptides, mostly Aβ 1-42, have a propensity for self-aggregation into fibrils that form the senile plaques characteristic of AD pathology. Subsequently, it is thought that the microtubule-associated Tau protein in neurons becomes abnormally hyperphosphorylated and forms NFTs that disrupt neurons. A hypothetical model for biomarker dynamics in AD pathogenesis has been provided. This model begins with the abnormal deposition of Aβ fibrils, as evidenced by a corresponding drop in the levels of soluble Aβ 1-42 in the cerebrospinal fluid (CSF) and increased retention of a positron emission tomography (PET) radioactive tracer [11C]-labeled Pittsburgh compound B (11C-PiB) in the cortex. Sometime later, neuronal damage begins to occur, as evidenced by increased levels of CSF Tau protein. Synaptic dysfunction follows, resulting in decreased [18F]-fluorodeoxyglucose uptake as measured by PET. As neuronal degeneration progresses, atrophy in certain areas that are typical of AD becomes detectable by MR.

Despite intensive research, the clinical and pathologic relationship between AD and glaucoma remains obscure. No consensus has been established regarding whether clinical correlations between the two diseases might be due to shared risk factors or the influence of one disorder on the other, and different mechanisms may trigger the biomolecular processes leading to cell death in these diseases. For example, cell death may occur due to endoplasmic reticulum stress, oxidative stress, high levels of nitric oxide, defective axonal transport, or glial cell pathology. In our experiment, AD-like pathology was present in the LGN. Aβ 1-42 also was expressed weakly in V1. However, in our experiment, no positive findings were detected in cognitive areas (particularly the Hpp) that are affected most severely in AD. With respect to this interesting finding, we hypothesize that IOP elevation produces axonal and synaptic changes that trigger the biomolecular processes that lead to cell death, during glaucoma progression, along the visual pathway in an ascending order, from the RGCs to the ONH, LGN, and even the visual cortex, ultimately resulting in an AD-like pathology.

The importance of early axonal and synaptic loss in neurodegenerative disease is being recognized increasingly.
Glaucoma involves the degeneration of RGCs and their axons in the optic nerve. RGC axons exit the eye and enter the optic nerve by passing through the ONH. The current hypothesis is that an initial and critical insult damages RGC axons in the ONH as they exit the eye. Higher IOP is an important risk factor for glaucoma, but the molecular links between elevated IOP and axon damage in the ONH are not understood. However, it is known that axonal transport impairment precedes axonal degeneration in glaucoma. Previous studies in monkeys and more recent studies in experimental rat models of glaucoma have shown that retrograde axonal transport in glaucoma models was blocked at the level of the ONH.

Synaptic dysfunction has been suggested as the initial pathologic change that leads to neuronal death in multiple progressive neuropathologic conditions of the central and peripheral nervous system, including AD. Collectively, these can be described as dying-back neuropathies or distal axonopathies that evolve from synaptic dysfunction, which is followed by target detachment and progression to neuronal death. Similar histopathologic changes also occur in non-ischemic remote brain regions with synaptic connections with the primary lesion site. For example, in the ipsilateral thalamus, Aβ was shown to accumulate abnormally and aggregate into plaque-like deposits for up to 9 months after middle cerebral artery occlusion. In addition, our observation that the distribution of AD-like pathology along the visual pathway occurred in an ascending order without being observed in cognitive areas, particularly the Hpp, indicates that IOP elevation may produce damage to the glaucomatous CVS via axonal and synaptic changes.

In summary, to our knowledge these data are the first to establish the existence of hallmark AD-like pathologies, such as Aβ 1-42 and p-Tau, in the glaucomatous LGN following injury to RGCs. In addition, the distribution of AD-like pathology along the visual pathway occurs in an ascending order, indicating that IOP elevation may produce damage to the glaucomatous CVS via axonal and synaptic changes. Briefly, our

**Figure 6.** Western blot and ELISA for Aβ 1-42 and p-Tau. (A) Western blot analysis reveals strongly positive Aβ 1-42 and p-Tau bands in glaucomatous LGN, and weakly positive Aβ 1-42 expression in glaucomatous V1. (B) Quantification of Aβ 1-42 and p-Tau, compared versus control normal tissue by Western blot. The densitometric signal for each sample was adjusted to β-actin, and the ratio of glaucomatous sample/control normal sample was calculated. (C, D) Quantification of Aβ 1-42 and p-Tau, compared versus control normal tissue by ELISA. The glaucomatous LGN and V1 sections exhibit significantly higher levels of Aβ 1-42 than controls. The data are expressed as nanograms Aβ 1-42 or p-Tau per milligram of brain tissues. Data for each group were averaged ± SEM (n = 3). **P < 0.01, *P < 0.05. (G, Glaucoma; C, Control).**
results supported the hypothesis that targeting the mechanisms of Aβ deposition or tauopathy may drive research into neuroprotective glaucoma therapies in a new direction.

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