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Proton magnetic resonance spectroscopy revealed choline reduction in the visual cortex in an experimental model of chronic glaucoma

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ABSTRACT

Glaucoma is a neurodegenerative disease of the visual system. While elevated intraocular pressure is considered to be a major risk factor, the primary cause and pathogenesis of this disease are still unclear. This study aims to employ *in vivo* proton magnetic resonance spectroscopy (^{1}H MRS) to evaluate the metabolic changes in the visual cortex in a rat model of chronic glaucoma. Five Sprague–Dawley female rats were prepared to induce ocular hypertension unilaterally in the right eye by photocoagulating the episcleral and limbal veins using an argon laser. Single voxel ^{1}H MRS was performed on each side of the visual cortex 6 weeks after laser treatment. Relative to the creatine level, the choline level was found to be significantly lower in the left glaucomatous visual cortex than the right control visual cortex in all animals. In addition, a marginally significant increase in glutamate level was observed in the glaucomatous visual cortex. No apparent difference was observed between contralateral sides of the visual cortex in T1-weighted or T2-weighted imaging. The results of this study showed that glaucoma is accompanied with alterations in the metabolism of choline-containing compounds in the visual cortex contralateral to the glaucomatous rat eye. These potentially associated the pathophysiological mechanisms of glaucoma with the dysfunction of the cholinergic system in the visual pathway. ^{1}H MRS is a potential tool for studying the metabolic changes in glaucoma *in vivo* in normally appearing brain structures, and may possess direct clinical applications for humans. Measurement of the Cho:Cr reduction in the visual cortex may be a noninvasive biomarker for this disease.

1. Introduction

Glaucoma is a neurodegenerative disease of the visual system characterized by retinal ganglion cell (RGC) death, optic nerve head damage, and progressive visual field loss (Thanos and Naskar, 2004). It is the second major cause of blindness in the world (Quigley and Broman, 2006). While elevated intraocular pressure (IOP) is considered a major risk factor, the primary cause to the atrophic processes is still unclear (Kaufman, 1999). Recently, increasing evidence has been found suggesting the dissemination of glaucomatous damage in the posterior visual pathway in relation to transsynaptic degeneration (Crawford et al., 2000; Duncan et al., 2007; Gupta et al., 2006; Gupta and Yucel, 2003, 2007; Lam et al., 2003; Parisi, 2001; Parisi et al., 2001; Yucel et al., 2003). It has also been demonstrated that exogenous cytidine 5′-diphosphocholine (CDP-choline, or citicoline), which is an intermediate in the generation of phosphatidylcholine (PtdCho) and acetylcholine (ACh) from choline (Cho), may improve visual cortical responses in glaucoma (Parisi, 2005; Parisi et al., 1999; Rejdak et al., 2003). Proton magnetic resonance spectroscopy (^{1}H MRS) has been increasingly utilized to investigate the metabolite distribution in selected volumes of the brain *in vivo* (Babb et al., 2004; Bianchi et al., 2003; Boulanger et al., 2000; Cordoba et al., 2002; Gomez-Anson et al., 2007; Kantarci et al., 2007; Wang et al., 2008; Xu et al., 2005). However, *in vivo* studies of the metabolic changes in human glaucoma are limited (Boucard et al., 2007), possibly due to limited sensitivity and spectral resolution at low magnetic field strengths, limited regional specificity and biological variations between experimental groups. In this study, an experimental glaucoma model was induced by laser photocoagulation of the episcleral and limbal veins in the rat eye, mimicking the pathogenesis of human primary open-angle glaucoma (Li et al., 2006b). As more than 98.5% of the RGC axons in rats decussate to the contralateral visual cortex at the optic chiasm (Fleming et al., 2006), the contralateral visual

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cortex receiving input from the treated eye can be compared to the ipsilateral visual cortex receiving input from the intact eye, minimizing biological variations from between-group comparisons. Given that the *in vivo* Cho resonance reflects the abundance of Cho, phosphocholine (PCho), glycerophosphocholine (GPC), ACh, and other Cho compounds (Dowling et al., 2001), this study employs *in vivo* high-field ^{1}H MRS to evaluate the metabolic changes in normally appearing brain structures in a rat model of chronic glaucoma. In particular, we aim to test the hypothesis that alterations in the metabolism of Cho-containing compounds may occur in the visual cortex in chronic glaucoma.

2. Methods

2.1. Animal preparation

Sprague–Dawley female rats (250–280 g, $N = 5$) were reared in a temperature-controlled room subjected to a 12 h light/12 h dark cycle with standard chow and water supply *ad libitum*. They were prepared to induce ocular hypertension unilaterally in the right eye by photocoagulation of the episcleral and limbal veins using an argon laser. A second laser treatment in the same settings was applied 7 days later to block the neovascular flow. This technique has been adopted in our laboratory for the study of retinal and optic nerve degeneration (Chan et al., 2008b, 2007; Hui et al., 2007; Li et al., 2006a,b) as well as ocular transport (Chan et al., 2008a,b, 2007). The IOPs of the glaucomatous and control eyes were measured using a calibrated tonometer (Tonopen-XL, Mentor, Norwell, MA, USA), and were found to be 13.50 ± 0.90 mmHg and 13.56 ± 0.57 mmHg respectively before laser treatments ($p = 0.93$), and 23.07 ± 2.15 mmHg and 13.42 ± 1.07 mmHg respectively after two laser treatments ($p < 0.001$). This IOP elevation by about 1.7 times above the normal level was shown to be maintained up to a maximum of 12-week experimental period (Li et al., 2006a,b). After each procedure, antibiotic ointment was applied topically to the eye surface. Six weeks after laser treatment, ^{1}H MRS was performed at the visual cortex. Throughout the experiments, the left eye and the right visual cortex served as the internal control.

2.2. MRI protocols

All MRI measurements were acquired on a 7 T MRI scanner with a maximum gradient of 360 mT/m (70/16 PharmaScan, Bruker Biospin GmbH, Germany) using a 72 mm birdcage transmit-only RF coil with an actively decoupled receive-only quadrature surface coil. The rat was placed onto a head holder comprising a tooth bar. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), the animal was kept warm under circulating water at 37 °C. Scout images were first acquired in three planes with a fast spin echo (FSE) sequence to position the subsequent multi-parametric MR images along standard anatomical orientations in a reproducible manner. 2D T1-weighted imaging (T1WI) using FSE sequence was acquired with FOV = 3.2 × 3.2 cm^2, matrix resolution = 256 × 256, slice thickness = 1 mm, number of slices = 10, TR/TE = 400/7.5 ms, echo train length = 4 and NEX = 16; T2-weighted imaging (T2WI) was performed under the same dimensions with TR/TE = 4200/38.7 ms, echo train length = 8 and NEX = 2; for ^{1}H MRS, a 4 × 1 × 4 mm^3 voxel was placed over each side of the visual cortex. The volume of interest was maximized to obtain higher signal-to-noise ratios and to cover the gray matter predominantly in the visual cortex, while avoiding the margins of the white matter structures, which were clearly distinguishable in T2WIs underneath the cortex. After first- and second-order localized voxel shimming with fast automatic shimming technique b mapping along projections (FASTMAP) (Gruetter, 1993), a full-width half-maximum linewidth of water signal of ≤20 Hz would be achieved. The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOR). Outer volume suppression (OVS) combined with point-resolved spectroscopy (PRESS) sequence was used for signal acquisition, with TR/TE = 2000/20 ms, spectral bandwidth = 4 kHz, 2048 data points and 128 averages.

2.3. Data analysis

The morphology in T1WI and T2WI was assessed qualitatively in the visual cortex between contralateral sides. Regions of interest (ROIs) were then drawn manually on each side of the visual cortex covered by the single voxel in ^{1}H MRS using ImageJ v1.40g (Wayne Rasband, NIH, USA) with reference to the rat brain atlas (Paxinos and Watson, 2007). Each value was calibrated to the nearby phantom containing saline solution to avoid the effect of any MRI system sensitivity drift.

The *in vivo* MR spectra were processed using the jMRUI software (version 3.0, http://www.mrui.uab.es/mrui/) (Ratiney et al., 2005). The signal of residual water was filtered with Hackel–Lanczos Singular Value Decomposition (HLSVD) algorithm preprocessing with 25 spectral components for modeling. In addition, a Gaussian apodization of 15 Hz was applied to increase the signal-to-noise ratio of the spectrum. Spectral peaks were assigned in the references of the singlet peak of NAA (CH_3-group). Metabolite areas were estimated using the quantitation based on quantum estimation (QUEST) method combined with subtraction approach for background modeling (Cudalbu et al., 2008). To reduce systematic variations among studied animals and to accurately extract the dominating metabolite changes, a relative quantification method using creatine (Cr) peak as the internal spectral reference was applied. The numerical time-domain modal functions of 10 metabolites [alanine (Ala), aspartate (Asp), Cr, Cho, glutamate (Glu), glycine (Gly), *N*-acetylaspartate (NAA), taurine (Tau), lactate (Lac) and myo-inositol (mI)] were used as prior knowledge in QUEST. These metabolite model signals were quantum mechanically simulated in NMR spectra calculation using operators (NMR-SCOPE) for the *in vivo* experimental protocol. NAA:Cr, Cho:Cr, Glu:Cr, Lac:Cr, and Tau:Cr ratios were statistically evaluated. The reliability of metabolite quantitation was assessed using the Cramer-Rao lower bounds (CRLB) (Cudalbu et al., 2008). An estimate was considered as relevant when the corresponding bound was found below 25% of the estimate. The mean of the relevant estimates and the corresponding error values were computed.

Unless otherwise stated, all data were presented as mean ± standard deviation (SD). Two-tailed paired Student's *t*-tests were performed between contralateral sides of all measurements. Results were considered significant when $p < 0.05$.

3. Results

Fig. 1 shows the typical T1WI and T2WI of the visual cortex. The normalized signal intensities of T1WI and T2WI were measured to be 0.155 ± 0.014 and 0.441 ± 0.016 respectively in the left glaucomatous visual cortex, and 0.158 ± 0.015 and 0.452 ± 0.021 respectively in the right control visual cortex. No statistically significant difference was observed between contralateral sides of the visual cortex in either T1WI or T2WI ($p > 0.05$). Fig. 2 shows the localization of voxel placements to the visual cortex, and the averaged spectra for ^{1}H MRS. The mean values and the corresponding standard deviations of the estimated metabolite ratios were reported in Table 1. In ^{1}H MRS, all 5 animals showed a lower Cho:Cr ratio in the left glaucomatous visual cortex than in the right control visual cortex by 41% ($p < 0.05$). Except for a higher Glu:Cr ratio in the glaucomatous visual cortex with marginal significance ($p = 0.09$), no apparent difference was observed in other

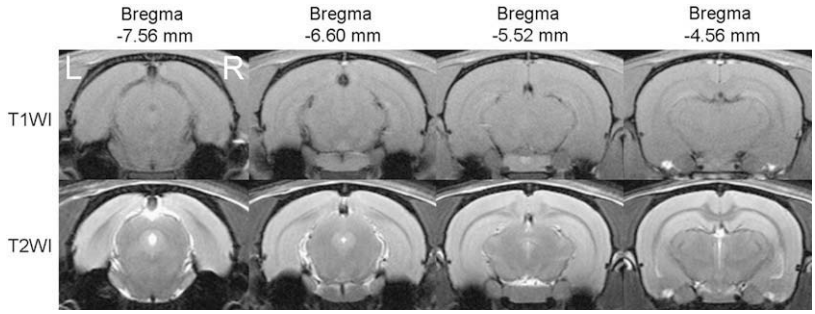

Fig. 1. Typical T1-weighted and T2-weighted images of the visual cortex at Bregma −7.56 mm to −4.56 mm. No apparent difference was observed between contralateral sides of the visual cortex.

metabolites between contralateral sides of the visual cortex ($p > 0.1$). Note that most of metabolites were quantified with CRLB of less than 25%.

4. Discussions

The results of the current study showed that glaucoma is accompanied with alterations in the metabolism of Cho-containing compounds in the rat visual cortex 6 weeks after induction of ocular hypertension. In the current glaucoma model, obstruction of aqueous humor outflow is the primary mechanism of pressure elevation analogous to the pathogenesis of human primary open-angle glaucoma. When a persistent elevation of IOP by 1.7 times was maintained in the rat eye of the same model, a 3% RGC loss per week was documented across the 8-week experimental period (Li et al., 2006a,b). Cho signal intensity reflects cytosolic Cho-containing compounds, 98% of which are PCho and GPC. They provide free Cho for the synthesis of the neurotransmitter ACh by choline acetyltransferase (ChAT), and for the storage in membranous PtdCho in cholinergic neurons (Boulanger et al., 2000; Kantarci et al., 2007; Michel et al., 2006; Schmidt and Rylett, 1993). The ACh level in different rat brain regions has been shown to correlate with the Cho signal intensity in ^{1}H MRS (Wang et al., 2008), while PtdCho is immobilized in cell membranes and is thus largely invisible to ^{1}H MRS (Miller, 1991). Reduction of Cho-containing compounds in the brain has been demonstrated in human and rodent ^{1}H MRS studies on mitochondrial diseases (Bianchi et al., 2003), Huntington disease (Gomez-Anson et al., 2007), hepatic encephalopathy (Cordoba et al., 2002; Taylor-Robinson et al., 1994), stable mild cognitive impairments (Kantarci et al., 2007), and after muscarinic agonist treatment in Alzheimer's disease (Satlin et al., 1997), and is shown to associate with less synthetic activity (Ende et al., 2001), impaired intraneuronal signaling mechanisms (Moore et al., 2000), decreased membrane turnover, and lower cellular density (Dowling et al., 2001). For glaucoma, it is likely the Cho:Cr reduction was a result of reduced cortical activity in addition to transsynaptic degeneration, and was partially associated with the dysfunction of the cholinergic system in the visual pathway.

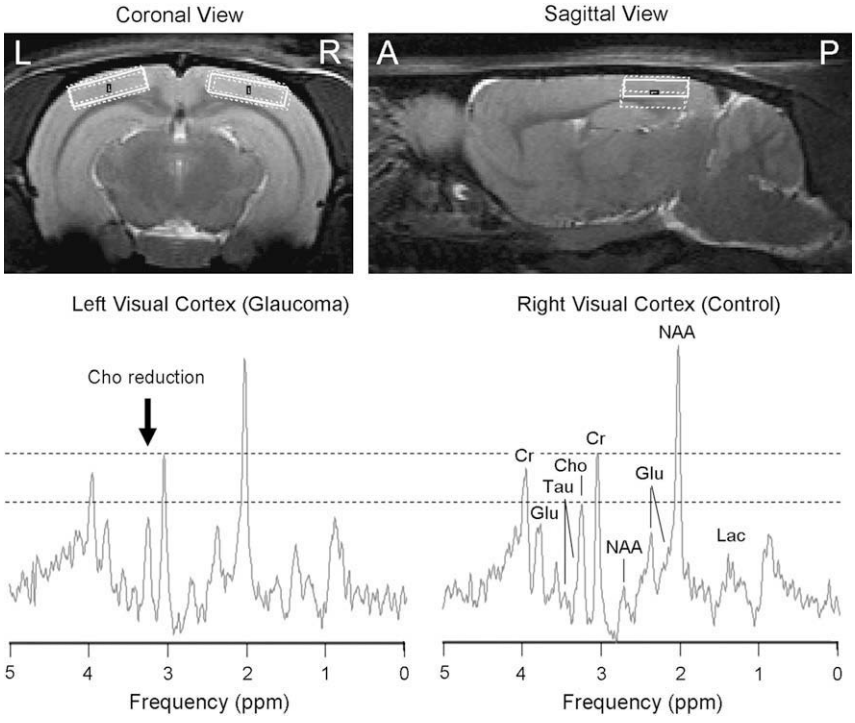

Fig. 2. (Top row) Illustration of the localization of the $4 \times 1 \times 4$ mm^3 voxels (solid-line boxes) in the glaucomatous (L) and control (R) rat visual cortex for ^{1}H MRS. (Bottom row) Averaged spectra for single voxel ^{1}H MRS on each side of the visual cortex. Note the apparently lower Cho signal (arrow) with respect to the Cr signal in the left glaucomatous visual cortex than in the right control visual cortex. (L: left; R: right; A: anterior; P: posterior.)

Table 1
Metabolite ratios and the respective Cramer-Rao lower bounds (CRLB) at each side of the visual cortex 6 weeks after glaucoma induction. (ns: not significant.)

Metabolites to creatine (Cr) ($N = 5$)	Left visual cortex (glaucoma)		Right visual cortex (control)		p Value (two-tailed paired t-test)
	Mean ± SD	Mean % SD (CRLB)	Mean ± SD	Mean % SD (CRLB)	
N-acetyl-aspartate (NAA)	1.36 ± 0.15	5.83 ± 0.38	1.28 ± 0.10	5.28 ± 0.76	ns
Choline (Cho)	0.16 ± 0.05	15.85 ± 4.81	0.27 ± 0.04	8.67 ± 0.92	<0.05
Glutamate (Glu)	1.44 ± 0.33	7.43 ± 1.63	0.96 ± 0.31	8.54 ± 1.89	ns (0.09)
Lactate (Lac)	0.52 ± 0.11	15.92 ± 4.03	0.38 ± 0.15	22.80 ± 8.88	ns
Taurine (Tau)	0.44 ± 0.13	16.84 ± 7.08	0.35 ± 0.24	19.02 ± 9.80	ns

Cholinergic neurons use Cho for the synthesis of the neurotransmitter, ACh, which plays a crucial role in synaptic transmission. In addition, release of ACh is a marker of activation of the primary visual cortex during visual stimulation (Fournier et al., 2004; Laplante et al., 2005). Recent functional MRI studies have shown the effect of visual field loss to the functional organization of human primary visual cortex in primary open-angle glaucoma (Duncan et al., 2007). Glaucomatous visually evoked potential abnormalities (Parisi, 2001, 2005; Parisi et al., 2001) and cytochrome oxidase activity decrease (Crawford et al., 2000; Lam et al., 2003; Yucel et al., 2003) in the primary visual cortex have also been observed and ascribed to impaired neural conduction along postretinal visual pathways, due to the dysfunction of the innermost retinal layers containing the RGCs and their fibers upon RGC loss and elevated IOP induced activity changes (Gupta and Yucel, 2003; Yucel et al., 2003). Using electroretinography, the laser-induced ocular hypertension model in the present study also showed a substantial reduction of retinal functions (Li et al., 2006b). Since the animals were exposed in their cages 12 h/day to light over a 6-week period following laser coagulation, the visual stimulation of the left and right cortex would differ over time. Although it has been reported that acute visual stimulations did not cause significant changes in MR spectra in the human occipital lobe (Boucard et al., 2005), it is possible that the long-term effect of the differences in visual stimulation and cortical activation in the treated and control hemispheres may contribute to the reduction in Cho level upon neurodegeneration of the visual system in the glaucoma rat model. The expression of cholinergic receptors in the visual cortex was found to be regulated by visual input activity and functional state of the visual cortex (Gu, 2003). Analogous to the effect of RGC loss in the treated eye on the contralateral visual cortex in the current model, the spontaneous ACh release from chronically undercut areas of mammal visual cortex was found to be considerably lower than from intact areas of cortex (Collier and Mitchell, 1967; Sato et al., 1987). The amount of ACh in visual cortex also significantly decreased at 2 and 4 weeks after unilateral orbital enucleation in adult rats (Kim et al., 1995). Decreased Cho-containing compounds in ^{1}H MRS in the frontal cortex of preclinical carriers of Huntington disease have been shown to correlate to neuropsychological deficits (Gomez-Anson et al., 2007). It has also been postulated that when the cellular requirement for free Cho for ACh synthesis was reduced, e.g. by applying a muscarinic cholinergic agonist, neuronal membrane breakdown and the concentration of membrane catabolic intermediates, such as GPC could be reduced, resulting in a decrease in the brain Cho resonance in ^{1}H MRS (Satlin et al., 1997). Similarly, in the presence of transsynaptic degeneration and less visual stimulation in glaucoma, it is possible the ACh synthesis and storage in the visual cortex would be reduced, leading to fewer requirements for free Cho, less membrane breakdown, and thus a lower Cho:Cr ratio as observed in the current study.

On the other hand, abnormalities in mitochondrial transport and their defective distribution, whether caused by anatomical constraints (Chan et al., 2008b; Hui et al., 2007) or energy depletion (Osborne et al., 2006) along the glaucomatous visual pathway, may lead to local functional and metabolic crises, including Cho reduction (Bianchi et al., 2003), in correlation to a possible impairment of the normal processes of membrane maintenance due to local energy depletion (Carelli et al., 2004). ChAT is the biosynthesis enzyme of ACh, and its concentration reflects the level of cholinergic activity (Wessler et al., 2003). ChAT is shown to undergo anterograde axonal transport along the rat visual pathway (Yasuhara et al., 2003). Research evidence suggests that glaucoma obstructs anterograde axonal transport in RGC axons at the optic nerve head (McKinnon, 2003). Our previous MRI studies also showed reduced anterograde axonal transport of manganese ions along the glaucomatous visual pathway (Chan et al., 2008b, 2007) in addition to reduced fractional anisotropy and increased radial diffusivity in the prechiasmatic optic nerve by diffusion tensor imaging (Hui et al., 2007) in the same experimental glaucoma model. In the contralateral somatosensory cortex, peripheral deafferentation of the sciatic nerve resulted in significant reductions of ChAT activity and high-affinity Cho uptake (Rothe et al., 1990). ChAT and ACh activities were also reduced in the cortex and striatum in human patients and transgenic mouse models of Huntington disease at young ages (Smith et al., 2006; Vetter et al., 2003), whereby Cho resonance was found to decrease in the frontal cortex in preclinical carriers of Huntington disease patients (Gomez-Anson et al., 2007). Reduced trabecular ChAT has been found in congenital glaucoma rats in association with IOP rise (Gatzioufas et al., 2008). Since ACh is mainly synthesized from ChAT, if there's any damage and blockade to the anterograde axonal transport of ChAT along the glaucomatous visual pathway, it is possible that less Cho-containing compounds would be recruited in the visual cortex.

In addition to the synthesis of ACh, cells use Cho as the precursor of certain phospholipids, e.g. PtdCho, for the major constituents of all biological membranes (Michel et al., 2006). The cells were shown to die by apoptosis when deprived of Cho, possibly due to interruption of cell cycling as a result of the decrease in membrane PtdCho concentration (Yen et al., 1999). In the presence of advanced human glaucoma with 50% visual field loss, neurodegeneration was observed in the visual cortex (Gupta et al., 2006). In the current study, there was no significant difference in NAA:Cr representing neuronal integrity between contralateral sides of the visual cortex. This appeared to relate to the fact that there was only approximately 20% of RGC loss being observed between week 4 and week 8 after glaucoma induction in the current model (Li et al., 2006a,b). While target cell loss represents perhaps the most extreme case of a reduction in trophic supply, decreases due to reduced synaptic connectivity or decreased lateral geniculate nucleus and visual cortex activity might also play an important role, especially during early stage of degeneration (Weber et al., 2008). Our results reflected the initial changes in Cho:Cr that may indicate subtle disturbances of neurological function preceding the development of overt glaucoma in the visual cortex.

Recently, it has been demonstrated that exogenous CDP-choline (citicoline) may improve visual cortical responses in patients with glaucoma, yet the mechanism of its actions in the visual system is not entirely understood (Parisi, 2005; Parisi et al., 1999; Rejdak et al., 2003). Once absorbed, citicoline is widely distributed throughout the body, undergoes a quick transformation to Cho and

cytidine, and crosses the blood–brain barrier into the central nervous system (Grieb and Rejdak, 2002; Secades and Lorenzo, 2006). Cho ingestion increases the *in vivo* ^{1}H MRS resonance of Cho-containing compounds in human brain (Babb et al., 2004). ^{1}H MRS also showed an increase in Cho resonance in the brain of young human subjects after single oral doses of CDP-choline (Babb et al., 1996). In addition to its neuroprotective properties on damaged RGCs in mouse culture retina (Oshitari et al., 2002), citicoline is known to increase in some brain areas the levels and enhance the rate of synthesis of ACh, dopamine, noradrenalin, and serotonin (Secades and Lorenzo, 2006). It is likely that a mechanism for citicoline to provide neuroprotective treatment to glaucoma is to normalize and replace the insufficient Cho contents in the glaucomatous visual cortex observed in the current study.

RGC loss in glaucoma has linked to programmed cell death, termed apoptosis. Among the initiating mechanisms described for RGC and optic nerve degeneration, both oxidative injury and glutamate excitotoxicity seen in neurodegenerative disease have been described in transsynaptic degeneration in primate glaucoma (Gupta and Yucel, 2007). In the etiology of glaucoma, inadequate supply of neurotrophic factors may synergize with excitotoxicity (Grieb and Rejdak, 2002). Similarly, Glu, another major excitatory neurotransmitter in the rat visual cortex (Baughman and Gilbert, 1980), is released by neurons upon stimulation, and is then taken up by surrounding glial cells and converted into glutamine by glutamine synthetase in astrocytes, leading to a decrease in Glu concentration (Xu et al., 2005). The marginally significant increase in Glu:Cr in the glaucomatous visual cortex than the control cortex appeared to associate with the fact that less stimulations occurred in the glaucomatous cortex causing less Glu uptake and conversion by glutamine synthetase. It may also be a result of decreased glutamatergic synaptic activity (Fonnum and Lock, 2004). Note that a significant decrease in Glu uptake and glutamine synthetase activity has been observed in the rat glaucomatous retina (Moreno et al., 2005). Worsening of neuropsychological function has also been shown to correlate with an increase in brain Glu/glutamine in hepatic encephalopathy in company with Cho reduction (Cordoba et al., 2002).

Recent studies have indicated that application of brain-derived trophic factor to both the eye and the visual cortex results in increased levels of RGC survival and function that exceed those seen following treatment of the eye alone (Weber et al., 2008). While previous studies on glaucoma focused on eye mainly, the results of the current study further support the recent issues on the need to investigate into the brain changes in glaucoma so as to look for better treatment effects to this disease. More importantly, this *in vivo* spectroscopic method can be readily translated to study human glaucoma and can have direct clinical applications. In the human brain, the cortical sheet contains both gray and white matters in the range of a few microliters, and is vulnerable to larger partial volume effect than the rat visual cortex in both ^{1}H MRS and proton chemical shift imaging (^{1}H CSI) using ordinary clinical MR scanners. Recent studies demonstrated the ability of ^{1}H CSI to quantify metabolite concentrations in mammalian brains at microliter resolution under high magnetic field strengths (Juchem et al., 2005; Mlynarik et al., 2008). Future experiments would possibly enable the differentiation of the effects on Cho concentrations in gray and white matters upon chronically elevated IOP in humans.

5. Conclusion

The results of this study showed that glaucoma is accompanied with alterations in the metabolism of Cho-containing compounds in the visual cortex. The lower Cho signal is potentially a result of reduced ACh release upon transsynaptic degeneration and visual cortical dysfunction. It may also reflect the compromise of the structural integrity of the neuronal membranes before apparent neuronal cell loss occurred. These indicated that the underlying pathophysiological mechanisms of glaucoma are partially associated with the dysfunction of the cholinergic system in the visual pathway. Since citicoline undergoes a quick transformation to Cho and cytidine after administration and crosses the blood–brain barrier into the central nervous system, it is likely that a mechanism for citicoline to provide neuroprotective treatment to glaucoma is to normalize and replace the insufficient Cho contents in the glaucomatous visual cortex. ^{1}H MRS is a potential tool for studying the metabolic changes in glaucoma *in vivo* in normally appearing brain structures, and may possess direct clinical applications for humans. Measurement of the Cho:Cr reduction in the visual cortex can be a noninvasive biomarker for this disease.

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