1H MRS of the Visual Cortex under Chronic Ocular Hypertension

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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 (1). While elevated intraocular pressure (IOP) is considered a major risk factor, the primary cause to the atrophic processes is still unclear. Recently, increasing evidence has been found suggesting the dissemination of glaucomatous damage in the posterior visual pathway in relation to transsynaptic degeneration (2). It has also been demonstrated that exogenous CDP-choline (citicoline) may improve visual cortical responses in patients with glaucoma (3). Since citicoline is an intermediate in the generation of acetylcholine (ACh) and phosphatidylcholine (PtdCho) from choline (Cho), while the *in vivo* Cho resonance in ^{1}H-MRS is known to reflect the abundance of Cho, phosphocholine (PCho), glycerophosphocholine (GPC), ACh, and other Cho compounds (4), this study aims to employ high-field ^{1}H-MRS to test the hypothesis that alterations in the metabolism of choline-containing compounds may occur in the visual cortex in a chronic glaucoma model.

METHODS:

Experimental Procedures: Sprague-Dawley female rats (250-280g, N=5) were prepared to induce ocular hypertension unilaterally in the right eye by photocoagulating the episcleral and limbal veins using an argon laser. ^{1}H MRS was performed at the visual cortex 6 weeks after laser treatment. Throughout the experiments, the left eye and the right visual cortex served as the internal control.

MR Imaging and Spectroscopy: All MR measurements were acquired utilizing a 7T Bruker PharmaScan 70/16 scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), animals were kept warm under circulating water at 37°C and were imaged using a receive-only surface coil. RARE T1WI and T2WI were acquired for morphological evaluations and subsequently for accurate placements of single voxels for ^{1}H-MRS. After shimming with FASTMAP, ^{1}H-MRS was performed using a PRESS sequence with TR/TE = 2000/20 ms and NEX = 128. A 4x1x4 mm^3 voxel was placed over each side of the visual cortex as shown in Figure 1.

Data Analysis: In T1WI and T2WI, regions of interest 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 a nearby saline phantom. For ^{1}H MRS, metabolite ratios were calculated and compared between contralateral sides of the visual cortex using the Sub-QUEST method in jMRUI (5). The numerical time-domain modal functions of 10 metabolites were quantum mechanically simulated in NMR-SCOPE and were used as prior knowledge in QUEST. NAA:Cr, Cho:Cr, Glu:Cr, Lac:Cr, mI:Cr and Tau:Cr ratios were statistically evaluated using Students' two-tailed paired t-tests as in Table 1. Results were considered to be significantly different when $p < 0.05$. Cramer-Rao lower bounds were below 25% of estimated amplitudes for most of the metabolites of interests quantitated.

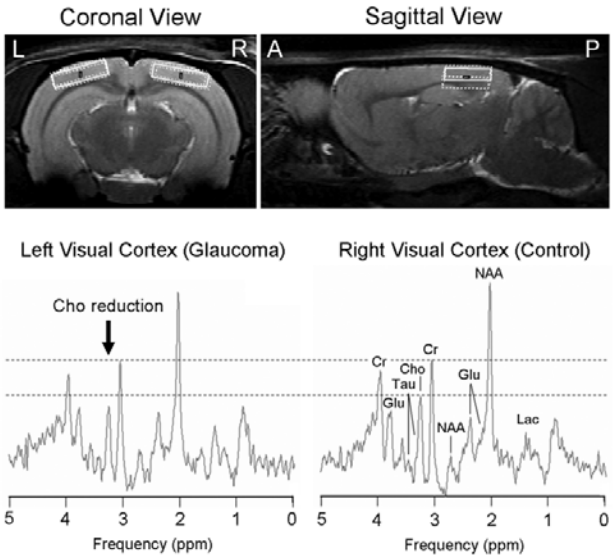

Figure 1: (Top) Illustration of the localization of the 4x1x4 mm^3 voxels to both sides of the visual cortex for ^{1}H-MRS. **(Bottom)** Averaged spectra for single voxel ^{1}H-MRS on each side of the visual cortex. Note the apparently lower Cho signal (arrow) with respect to the Cr signal in the left glaucomatous visual cortex than in the right control visual cortex.

Metabolites to Cr (N=5)	Left Visual Cortex (Glaucoma) Mean ± SD	Right Visual Cortex (Control) Mean ± SD	p value (Two-tailed Paired t-test)
NAA	1.36 ± 0.15	1.28 ± 0.10	ns
Cho	0.16 ± 0.05	0.27 ± 0.04	<0.05
Glu	1.44 ± 0.33	0.96 ± 0.31	ns (0.09)
Lac	0.52 ± 0.11	0.38 ± 0.15	ns
Tau	0.44 ± 0.13	0.35 ± 0.24	ns

Table 1: Metabolite ratios of both sides of the visual cortex 6 weeks after laser treatment. (ns: not significant.)

RESULTS:

The IOP of the glaucomatous eye was measured to be elevated by 1.7 times after laser treatment compared to the control eye ($p<0.001$). No statistically significant difference was observed between contralateral sides of the visual cortex in either T1WI or T2WI ($p>0.05$). In ^{1}H-MRS, all the 5 animals showed a lower Cho:Cr ratio in the left glaucomatous visual cortex than in the right control visual cortex ($p<0.05$). 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 metabolites between contralateral sides of the visual cortex ($p>0.1$).

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 rat eye of the same model, a 3% RGC loss per week was documented across the 8-week experimental period (6).Cho signal intensity reflects cytosolic Cho-containing compounds, 98% of which are PCho and GPC, which provide free Cho for the synthesis of the neurotransmitter ACh by choline acetyltransferase (ChAT), and for the storage in membranous PtdCho in cholinergic neurons. ACh is released in the rat primary visual cortex during visual stimulation (7), and its level in different rat brain regions correlates with the Cho signal intensity in ^{1}H MRS (8). The observed lower Cho signal is potentially a result of reduced ACh release upon transsynaptic degeneration (2) and prolonged visual cortical dysfunction (9) in glaucoma. 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 (10), it is also 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.

CONCLUSION:

^{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.

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