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Longitudinal non-invasive proton NMR spectroscopy measurement of vitreous lactate in a rabbit model of ocular hypertension

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Abstract

To determine whether vitreous lactate concentrations are correlated with intraocular pressure (IOP) rise, retinal ganglion cell (RGC) damage, and nerve fiber layer (NFL) thickness decrease in a rabbit model of ocular hypertension. Also, to learn whether proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy can provide sequential, non-invasive in vivo measurements of vitreous lactate. Intra-anterior chamber injections of 20-μm latex beads were used to impede aqueous drainage in New Zealand White rabbits, causing an elevation of IOP. Group I consisted of 12 rabbits in which unilateral elevations in IOP were achieved. Group II consisted of 6 rabbits in which treatment did not cause a change in IOP. The contralateral eye served as a control in both groups. Control eyes received an equal volume injection of vehicle only. IOP was measured for two pre-treatment days and then on post-treatment days 1, 3, 5, 7, 9, 16, 23, 30, and 37. ^{1}H-NMR spectroscopy was used to measure changes in vitreous lactate concentrations that may be associated with the onset and progression of the pathophysiology. Post-mortem histochemical analysis at the light microscope level was used to quantify changes in the retinal NFL thickness and in the numbers of RGC, and correlate them with IOP and percent change in lactate levels. Baseline IOP in Group I control and treated eyes were 12.0 ± 1.9 and 12.5 ± 1.3 mmHg, respectively. Between days 5 and 9 post-treatment, the IOP in Group I treated eyes ($n = 12$) rose to 23.9 ± 4.2 mmHg. IOP in the control eyes remained unchanged (12.0 ± 1.6). Vitreous lactate levels in Group I treated eyes increased by 100%, from pre-treatment values. Levels in control eyes remained unchanged. In Group I, IOP and percent change in lactate concentration in treated eyes were closely correlated throughout the study period ($r = 0.95$; $p \leq 0.05$). Group II control and treated eyes showed no significant changes in either IOP or lactate. Group I treated eyes had a reduced NFL thickness (65 ± 4 μm; $n = 5$) at the temporal medullary ray (MR) compared with control eyes (45 ± 6 μm). A smaller reduction was found in the nasal MR areas, where thickness was 53 ± 3 μm in treated eyes and 66 ± 4 μm in control eyes. RGC numbers also were decreased in the treated eyes (241,222 ± 10,920 cells) vs. 322,311 ± 8726 cells in control eyes. TdT-mediated dUTP nick-end labeling (TUNEL) indicated that RGC loss in the treated eyes was most likely due to apoptosis. In vivo changes in lactate can be monitored non-invasively over time using ^{1}H-NMR spectroscopy. Vitreous lactate concentrations increased and returned to baseline concurrently with IOP. The brief elevation in IOP produced a reduction in both the RGC cell numbers and in the thickness of the NFL.

Keywords: intraocular pressure; ocular hypertension; rabbit model; retinal ganglion cell; nerve fiber layer; vitreous lactate; non-invasive; proton nuclear magnetic resonance spectroscopy

1. Introduction

Relative to other neuronal tissue, the retina produces a large amount of lactate during glucose metabolism (Winkler, 1981). It has been proposed that the cellular events underlying various retinopathies, such as diabetic retinopathy, may involve perturbations of retinal glucose metabolism (Berkowitz et al., 1995). Vitreous lactate concentrations have been found to be significantly elevated in glaucoma eyes compared to normal controls, and the degree of elevation appeared to be correlated with advancing optic nerve damage (Lee et al., 2001). Also immunohistochemical studies have found an

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increase in the concentration of lactate and several other metabolites when IOP is increased (Sperber and Bill, 1985; Yang et al., 1995).

Changes in the rate of retinal glucose metabolism can be quantified by measuring either glucose consumption or lactate production. Berkowitz et al. (1995) showed that steady-state vitreous lactate could be measured non-invasively in rabbits using ^{1}H-NMR spectroscopy. The present study investigated whether the same technique could be used to monitor longitudinal changes in lactate levels in the rabbit model of ocular hypertension.

^{1}H-NMR spectroscopy has been used extensively to study the normal and diseased human brain and as a tool to evaluate the effects of neuroprotective agents in brain ischemia (Laptook et al., 1995a,b). Human (Rucker et al., 2003) and rabbit (Berkowitz, 1994) vitreous lactate concentrations are high enough in normal eyes to be measured using this technique. Furthermore, there are indications from preliminary studies that vitreous levels of lactate are elevated in primary open angle glaucoma (Lee et al., 2001). Non-invasive measurements of vitreous lactate might provide a biochemical marker associated with elevations in IOP in experimental rabbit ocular hypertension, and might signal that retinal damage is occurring in this animal model.

The purpose of this study was to evaluate the ability of single-voxel ^{1}H-NMR spectroscopy to detect and monitor changes over time in the levels of vitreous lactate in a rabbit model of ocular hypertension in comparison to control eyes. In addition the study asked whether changes in lactate levels are correlated with changes in IOP and may indicate that retinal damage is occurring during the elevated IOP.

2. Materials and methods

2.1. Animals and induction of ocular hypertension

New Zealand White rabbits were obtained from Myrtles Rabbitry (Thompson Station, TN.) 2–3 weeks after birth. Rabbits were anesthetized by an intramuscular injection of 34 mg/kg ketamine (Phoenix Scientific, Inc., St. Joseph, MO) and 34 mg/kg xylazine (Ben Venue Laboratories Inc., Bedford, OH). This dosage sedated the animals for approximately 1 h. Topical anesthesia was achieved by application of 1 drop 0.5% proparacaine HCl (Allergan Ophthetic) onto the cornea of each eye. A single intra-anterior chamber injection of latex beads was used to induce ocular hypertension. Following the method of Weber and Zelenak (2001) a 50-mL volume of 20 μm sterile latex beads (Coulter Corporation, Miami, FL) was injected through the cornea and into the anterior aqueous chamber using a micro-syringe and a 30-gauge needle. Hemacytometer (Cambridge Instruments, Buffalo, NY) counts of latex beads were 425,000 ± 35,355 particles/50 ml volume of the suspension. Contralateral control eyes in all animals had an equivalent volume of the sterile vehicle (normal saline) injected into the anterior chamber. Of 18 treated rabbits, 12 satisfied the study criteria of a sustained elevation in IOP over several days after a single injection of the latex beads into the anterior chamber (Group I). IOP levels did not change in the remaining 6 animals (Group II). Lactate and IOP values were obtained from both Group I and Group II rabbits. Six rabbits from Group I were reserved for histological examination of ganglion cell populations. Five of these rabbits were also used for nerve fiber layer (NFL) thickness analysis.

2.2. ^{1}H-NMR data collection and analysis

Initial experiments were conducted in which vitreous lactate concentrations in normal control animals were measured against a lactate solution (0.1 ml, 10 M solution) attached to the outer surface of the coil's eyepiece to assess the levels of normal vitreous lactate. The calculated concentration was compared to that obtained when using vitreous water as the standard. There was no significant difference between the two measurements. We, therefore, decided to use only the vitreous water standard to streamline the protocol. All spectral acquisitions were done at the University of Alabama at Birmingham (UAB) Center for Nuclear Imaging Research in the Department of Medicine. Measurements were performed using a 4.7-Tesla 30-cm horizontal bore magnet (Bruker Instruments, Billerica, MA) running Paravision 3.0 software. Animals were positioned in a non-metallic customized holder such that either the right or left eye was located within 2 cm of the isocenter. A 2-cm surface coil tuned to 200.48 MHz was positioned over the eye. Gradient echo images were taken in the horizontal, sagittal, and coronal dimensions for positioning of the volume to be measured (Fig. 1). The volume of interest (voxel) was typically 0.21 cc (3 × 8.4 × 8.5 mm) taken near the center of the vitreous chamber to avoid significant contamination from the periorbital fat, lens, and aqueous humor. Shimming was carried out on the voxel to correct for any inhomogeneity in the magnet, and to adjust the power to give the maximal signal. Localization was achieved by using a water suppressed Point Resolved Spectroscopy (PRESS) sequence, TE = 68 ms, SW = 2 kHz, and a chemical shift offset of −660 Hz to coincide with the lactate resonance. A representative lactate spectrum after water suppression is shown in Fig. 2. The number of averages was 320 collected in five blocks of 64 with a repetition time of 2.5 s. This scheme was used in case the animal woke up during the acquisition to allow rejection of the data following the movement. Each block was inspected prior to analysis. To confirm the identity of the resonance, a second acquisition of 64 averages was obtained with TE = 135 to achieve an inverted resonance due to J-coupling (not shown).

As a control for the concentration of water in the vitreous sample, an unsuppressed spectrum of 16 averages was also obtained. Data was analyzed on a SunBlade100 (Sun Microsystems, Mountain View, CA) using Sunspec software (Philips Medical Systems). Areas under the unsuppressed water resonance and the lactate resonance were measured and the concentration of lactate was calculated. This was done assuming the concentration of water to be 110 M in protons and taking into account differences in the longitudinal relaxation time