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ORIGINAL RESEARCH

Multi-Voxel 1H-MRS in Metachromatic Leukodystrophy

Mitra Assadi[1], Dah-Jyuu Wang[2], Yadira Velazquez-Rodriquez[3] and Paola Leone[4]

[1]Departments of Neurology and Pediatrics, Robert Wood Johnson Medical School. [2]Department of Radiology, University of Pennsylvania. [3]Department of Neurology, Rowan Medical School. [4]School of Osteopathic Medicine, Rowan University. Corresponding author email: massadi@capitalhealth.org

Abstract: Metachromatic leukodystrophy (MLD) is characterized by the accumulation of sulfatide sphingolipids in the brain and peripheral nerves. We report metabolite alterations recorded using multi-voxel proton spectroscopy of the brain in four children with MLD. The data revealed elevated myoinositol/creatine and lactate/creatine ratios as well as decreased N-acetyl aspartate/creatine ratios. We propose that elevation in myoinositol and lactate are caused by astrocytic gliosis and may be used as biomarkers for disease progression in MLD.

Keywords: MLD, lactate, mI, multi-voxel ^{1}H-MRS

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Introduction

Metachromatic leukodystrophy (MLD) is a severe neuro-metabolic disorder caused by a deficiency in the lysosomal enzyme Arylsulfatase A which catalyzes degradation of 3-O-sulfogalactosyl-ceramide, an essential and abundant component of myelin.[1] In MLD, the storage of sulfatides in the oligodendrocyte and Schwann cells causes progressive demyelination in the central and peripheral nervous systems.[2] Due to the pervasive nature of this disease, metabolic alterations in MLD may go beyond sulfatide accumulation and involve other metabolites. Thus far, there have been limited studies of ^{1}H-magnetic resonance spectroscopy (MRS) of the brain in MLD. Bizzi et al implemented a long echo-time (TE) ^{1}H-MRS in 70 patients in an attempt to classify the patterns of metabolite abnormalities in various types of leukodystrophies.[3] Kruse et al studied seven cases of MLD using short TE ^{1}H-MRS and found a marked decrease in the neuronal marker N-acetyl aspartate (NAA), and elevated myo-inositol (mI) levels.[4] Multi-voxel ^{1}H-MRS can be used to examine large areas of the brain and therefore is an efficient tool for researching MLD, which causes diffuse cerebral changes without any focal lesions. Previous studies have implemented short TE ^{1}H-MRS studies, which can be used to evaluate many different brain metabolites. Although long TE studies are limited in the type of spectra that can be acquired, they do improve the spectral resolution for certain metabolites. As such, we studied metabolite abnormalities in MLD using multi-voxel long TE ^{1}H-MRS of the brain.

Methods

Four patients with clinical, laboratory, and genetic confirmation of MLD were recruited into a recently published treatment trial.[5] All patients had the late infantile form of MLD and were advanced in their disease. Table 1 summarizes the genotypic and phenotypic characteristics of the cohort. The subjects underwent brain imaging studies according to the previously described protocol. Long TE multi-voxel ^{1}H-MRS was implemented to measure changes in selected metabolites without contamination by lipid signals. Studies were conducted with a Siemens Magnetom Avanto 1.5 Tesla MRI system (Munich, Germany). An area of interest measuring $8 \times 8 \times 1.5$ cm^3 was marked in the center of the brain and included the bilateral basal ganglia, thalami, white matter, ventricles, and parts of the cortex (Fig. 1). Using a spin-echo (PRESS) sequence with TR/TE of 1700/135 msec and a spectral width of 1 KHz, 1024 complex data points and 4 averages were acquired. Two-dimensional phase-encoding (16×16 matrix, 16.0×16.0 cm^2 FOV) with a slice thickness of 1.5 cm was utilized to produce consecutive voxels measuring 1.5 cm^3 each throughout the area of interest. Spectral peak areas were analyzed using the LCModel program installed on a Linux-based 3.8 GHz Intel-Xeon image server and analysis workstation.[6]

Multi-voxel ^{1}H-MRS was used to measure metabolite ratios of NAA, mI, choline (Cho), and lactate (Lac) to creatine (Cr) in consecutive voxels in the area of interest. This method does not provide accurate metabolite concentrations, although the metabolites can be reported as ratios to Cr. All data were compared to age-matched control subjects who had been previously studied in our lab.

Results

Figure 2 is an example illustration of the spectral map acquired for one of the patients. Figure 3 demonstrates a sample of the spectra acquired from subject 1 at the level of the peri-ventricular white matter. The inverted lactate peaks were acquired at 1.3 parts per minute.

The data demonstrated mildly elevated Lac/Cr ratios. The highest Lac/Cr ratios were noted in the peri-ventricular region as illustrated in Figure 4. In order to better understand the value of this finding,

Table 1. Genotypic and phenotypic manifestations in the study cohort.

Cases	Age	Mutations	Delayed development	Seizures	Urine sulfatides	Neuropathy
Case1	4 years	ARSA gene	Severe	+	>20 times normal	+
Case 2	5 years	SAP B gene	Severe	–	>20 times normal	+
Case 3	4 years	SAP B gene	Severe	–	>20 times normal	+
Case 4	3 years	ARSA gene	Severe	–	>20 times normal	+

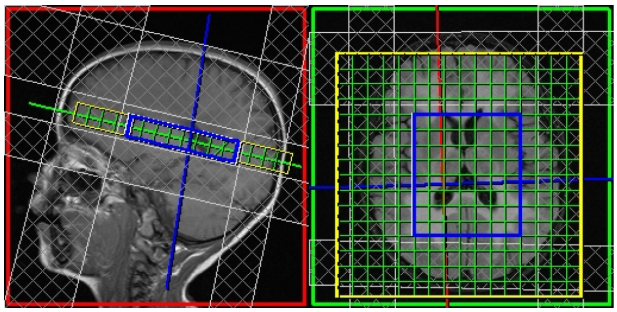

Figure 1. Area of interest in subject 1, two-dimensional representation of the volume of interest, marked by the blue box with size of 7 (R/L) × 8 (A/P) × 1.5 (F/L) cm^3.

we compared the ratios to those of four age-matched controls previously studied in our lab. These were developmentally normal children who had been evaluated for headaches (control subjects 1 and 2) or seizures (control subjects 3 and 4). Figure 5 demonstrates the mean (calculated in 3 voxels) of the Lac/Cr ratios in the subjects and the age-matched controls at the level of the peri-ventricular matter. The mean of the Lac/Cr ratios in MLD cases was 0.42 compared to 0.11 in the control subjects. Statistical analysis confirmed a significant difference between the mean values (*t*-test, $P < 0.01$).

Table 2 shows the acquired NAA and mI metabolite ratios from selected voxels in the four MLD cases and the age-matched control subjects. The NAA/Cr ratios were significantly reduced in the white matter (*t*-test, $P < 0.0001$) and the BG (*t*-test, $P < 0.0001$) in the MLD patients compared to controls. In contrast, the mI/Cr ratios were markedly elevated in the

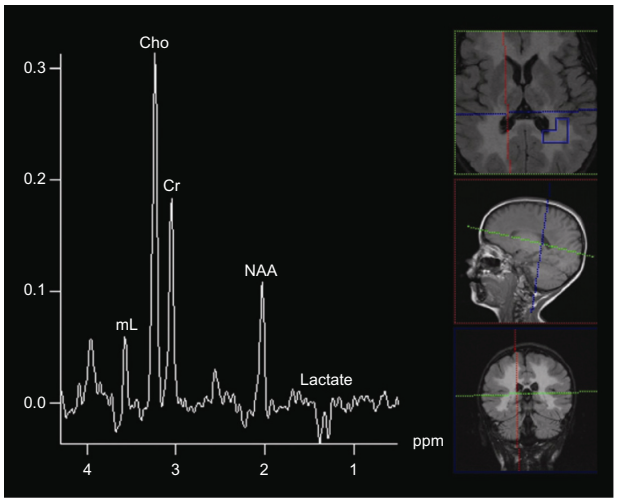

Figure 3. Three voxel average of the spectra acquired in subject 1 at the level of the peri-ventricular white matter (TR = 1700 msec, TE = 135 msec). Metabolite peaks are labeled as follows: Lactate at 1.3 ppm, NAA at 2.0 ppm, Cr at 3.0 ppm, Cho at 3.2 ppm, and mI at 3.5 ppm.

BG (*t*-test, $P < 0.01$) and the white matter (*t*-test, $P < 0.0001$) in our subjects compared to normal controls.

We also noted a slight elevation in the Cho/Cr ratio, with peak levels at the basal ganglia as shown in Figure 6.

Discussion

The energy metabolism in the human brain has not been fully elucidated. In the past, direct absorption of glucose from the blood was considered to be the sole source of energy for the brain. In 1994, however, Pellerin and Magistretti[7–10] introduced the concept of

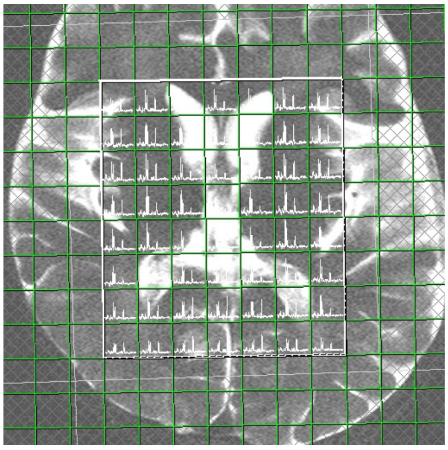

Figure 2. Spectral map recorded in the area of interest in subject 3, multi-voxel acquisition with 4 signal averages and a TR of 1700 msec and TE of 135 msec.

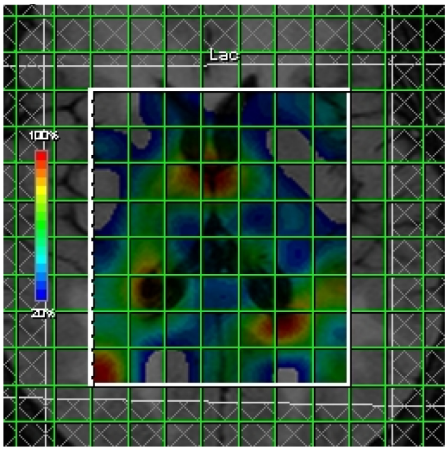

Figure 4. Lactate metabolite map in subject 1 (multi-voxel study with a TR/TE of 1700/135 msec). The peak lactate levels are observed in the peri-ventricular region and depicted by the red color.

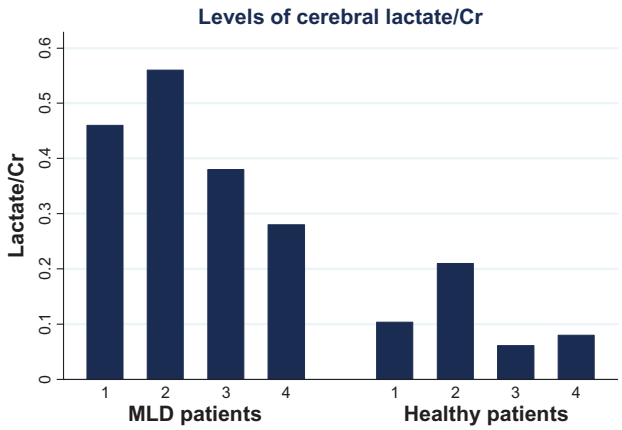

Figure 5. Mean of the lactate/Cr ratios in subjects and the age-matched controls. Average values were calculated from three voxels at the level of the peri-ventricular white matter.

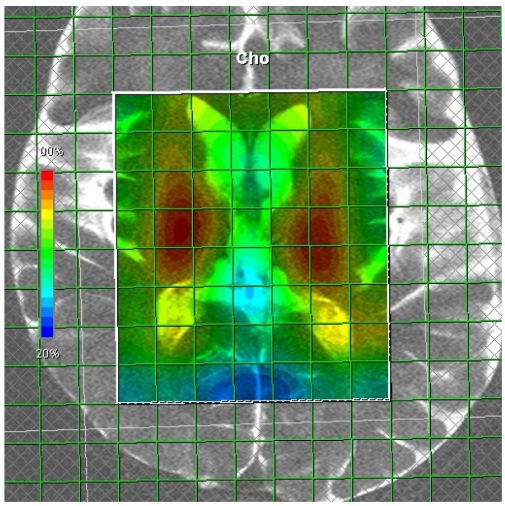

Figure 6. Choline metabolite map in subject 2 (multi-voxel study with a TR/TE of 1700/135 msec). The color red depicts the area with the highest concentration of the metabolite, which is in the basal ganglia.

"astrocyte-neuron metabolite trafficking". According to this model, which has been verified by additional studies,[11,12] glucose crosses the blood-brain barrier, entering the astrocytes to produce lactate via glycolysis. Subsequently, lactate is transported to the neurons where it enters the Krebs cycle in order to generate ATP. Glycolysis, and therefore lactate production, is enhanced by synaptic activation, which necessitates the reuptake of glutamate by astrocytes. This phenomenon is attributed to the fact that glutamate reuptake is an energy-dependent process and utilizes ATP.

Table 2. Samples of the acquired metabolite ratios in the peri-ventricular white matter and basal ganglia in the 4 subjects and 4 age-matched control.

Cases	Voxels	NAA/Cr	mI/Cr
1	PVWM 1	0.59	3.61
	PVWM 2	0.73	3.00
	BG	0.95	2.28
2	PVWM 1	0.83	3.94
	PVWM 2	0.80	4.08
	BG	1.17	2.49
3	PVWM 1	0.77	3.87
	PVWM 2	0.81	3.44
	BG	0.90	2.82
4	PVWM 1	0.37	4.32
	PVWM 2	0.23	3.22
	BG	0.95	2.61
Control 1, 6 y/o	PVWM 1	2.26	1.81
	PVWM 2	2.19	1.97
	BG	2.02	1.21
Control 2, 3.5 y/o	PVWM 1	1.87	1.75
	PVWM 2	2.30	1.84
	BG	2.59	1.52
Control 3, 4 y/o	PVWM 1	2.22	1.33
	PVWM 2	1.69	1.86
	BG	1.99	2.03
Control 4, 2.5 y/o	PVWM 1	1.71	0.93
	PVWM 2	1.51	1.74
	BG	2.43	0.89

Abbreviations: PVWM, peri-ventricular white matter; BG, basal ganglia.

This study demonstrates a mild diffuse elevation of the Lac/Cr ratios documented on multi-voxel ^{1}H-MRS in MLD patients which was statistically significant when compared to the controls. Bizzi et al[3] reported elevated lactate levels using single-voxel ^{1}H-MRS of children with various types of leukodystrophies. They used long TEs of 136 msec and successfully produced inverted lactate peaks which were easily distinguished from lipid signals. In their small MLD case series, Kruse et al also reported elevated lactate levels in seven MLD patients using ^{1}H-MRS with short TEs of 20 msec. The major limitation of their study was use the of short TEs, which is of limited utility in measuring lactate due to contamination caused by lipid signals. However, long TEs are not ideal for measuring other metabolites such as NAA and mI. To resolve this issue, we implemented a combination of long TEs and LCModel, enabling measurement of these metabolites.

Oligodendrocytes are known to support the axons through multiple mechanisms independent of myelination,[13] including their contribution to axonal energy supply. Oligodendrocytes possess the ability to transport lactate into axons. The most abundant lactate transporter, monocarboxylate transporter 1,

is known to be highly expressed in oligodendrocytes. Oligodendrocyte injury, which is well-documented in leukodystrophies, limits lactate transport into axons, which contributes to accumulation of this metabolite. In contrast, astrocytic gliosis in MLD causes an increase in lactate production as a result of increased cell mass. Conceivably, lactate uptake by the neuronal mitochondria is compromised in MLD due to progressive neuronal loss. It can be concluded that the elevation in the cerebral lactate is caused by a combination of increased production due to astrocytic gliosis, decreased transportation due to oligodendrocyte injury, and diminished utilization due to neuronal loss.

In addition to MLD, elevated mI levels have been described previously in Alzheimer's dementia.[14] This metabolite is generated by astrocytes and therefore an elevated mI level has been attributed to the histopathological finding of reactive gliosis characteristic of MLD and many other neurodegenerative diseases.[4] The elevation in the Cho/Cr ratio reflects a high myelin turnover and has been reported by several studies for MLD and other leukodystrophies.

The decrease in the NAA/Cr ratio is related to diffuse neuronal loss and, therefore, is not unexpected in MLD.[4] Dali et al[15] recoded ^{1}H-MRS of brain in 13 cases of late infantile MLD using intermediate TE of 99 msec. They reported a significant decrease in NAA levels, which strongly correlated with motor function, suggesting that NAA may be a useful marker for disease progression in MLD.

The results of this study validate our previous observations on short TE single-voxel ^{1}H-MRS in the same subjects,[5] showing low NAA/Cr and high mI/Cr ratios.

The major limitation of our study is the small sample size, caused by the scarcity of MLD cases. Fortunately, we were able to compare our results to those of four age-matched controls and demonstrated statistical significance. Although our sample size was small, the findings are robust and interesting.

We propose that elevated Lac/Cr and mI/Cr ratios are both attributable to the astrocytic gliosis characteristic of MLD. These markers, along with decreased NAA/Cr, are potentially useful for monitoring disease progression in MLD. To further confirm these findings, longitudinal studies of MLD patients over 1–2 years are necessary. These markers may guide future studies striving toward enzyme replacement therapy.

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Author Contributions

Conceived and designed the experiments: PL. Analyzed the data: DJW, PL, MA. Wrote the first draft of the manuscript: MA. Contributed to the writing of the manuscript: DJW, YV, PL. Agree with manuscript results and conclusions: MA, YV, DJW, PL. Jointly developed the structure and arguments for the paper: MA, DJW. Made critical revisions and approved final version: MA. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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