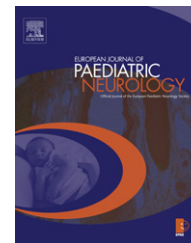




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Original article

Lithium citrate reduces excessive intra-cerebral N-acetyl aspartate in Canavan disease

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ABSTRACT

Our group has previously reported the first clinical application of lithium in a child affected by Canavan disease. In this study, we aimed to assess the effects of lithium on N-acetyl aspartate (NAA) as well as other end points in a larger cohort. Six patients with clinical, laboratory and genetic confirmation of Canavan disease were recruited and underwent treatment with lithium. The battery of safety and efficacy testing performed before and after sixty days of treatment included Gross Motor Function Testing (GMFM), Magnetic Resonance Imaging (MRI) Proton Magnetic Spectroscopy (H-MRS) as well as blood work. The medication was safe without any clinical or laboratory evidence for toxicity. Parental reports indicated improvement in alertness and social interactions. GMFM did not show statistically significant improvement in motor development. H-MRS documented an overall drop in NAA which was statistically significant in the basal ganglia. T1 measurements recorded on MRI studies suggested a mild improvement in myelination in the frontal white matter after treatment. Diffusion Tensor Imaging was available in two patients and suggested micro-structural improvement in the corpus callosum. The results suggest that lithium administration may be beneficial in patients with Canavan disease.

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1. Introduction

Canavan disease is an autosomal recessive leukodystrophy caused by mutations in the aspartoacylase (ASPA) gene. ASPA catalyses hydrolysis of N-acetyl aspartate (NAA) to acetate and aspartate. Consequently, the substrate molecule NAA, is

accumulated in the brain and causes a progressive neurodegenerative disorder characterized by dysmyelination and spongiform encephalopathy.¹ Clinically, the disease manifests as delayed development and/or developmental regression, significant axial hypotonia, macrocephaly, spastic diplegia, seizures and visual impairment.

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NAA is manufactured in the neuronal mitochondria and then transported to the extra-cellular fluid (ECF) and subsequently, to the oligodendroglial cells where it is catabolized to acetate and aspartate by ASPA.² In normal individuals, NAA is found exclusively and abundantly in the neurons. In Canavan disease, however, the excessively accumulated NAA overflows to the ECF and is absorbed into the blood stream resulting in mild metabolic acidosis. The NAA is then excreted in the urine causing mild aciduria. Marked elevation of NAA is the main biochemical hallmark of Canavan disease. Previous work has established that NAA increases linearly as a function of age in Canavan patients, with a frontal to occipital gradient which parallels the progression of clinical symptoms and spongiform degeneration.³ Although the exact mechanism of neuronal and white matter degeneration remains obscure, it is widely presumed to be caused by NAA elevation as well as metabolic disruption of myelin synthesis.^{4,5} A recent study supporting the acute toxic effects of NAA reported that intra-cerebral administration of the substance in rats causes protein oxidation in the cerebral cortex.⁶

The rationale for administering lithium citrate in Canavan disease derives from preliminary results reported from the animal studies. O'Donnell et al. demonstrated a significant drop of 9% in the brain NAA level in wild type rats after two weeks administration of lithium.⁷ Baslow observed a 13% reduction in the whole brain NAA in the tremor rat model of Canavan disease after only four days of intra-peritoneal lithium injection.⁸ Furthermore, lithium has been reported to have neuroprotective effects in patients affected by Alzheimer's disease and other dementias.^{9–11}

We have previously reported the first clinical application of lithium citrate in a child with Canavan disease¹² which suggested that lithium may reduce the typical increase of the NAA compared to the collected data on the natural history of the disease. Moreover, the patient showed a trend toward a normal pattern in the other imaging parameters.

This work, reports the findings of an open label trial of six additional patients with Canavan disease undergoing the previously reported study protocol.

2. Materials and methods

Six patients with Canavan disease were recruited according to the protocol described previously.¹² Table 1 summarizes the phenotypic and genotypic findings in the study cohort. Lithium dosage was escalated over a one-week titration to a total of 45 mg/kg/day. All patients received liquid lithium citrate (8 mEq/5 ml) manufactured by Roxan laboratories.

Brain Magnetic Resonance Imaging (MRI) and Proton Magnetic Resonance Spectroscopy (H-MRS) studies were

performed at baseline and after sixty days of treatment according to the previously reported imaging protocol.¹³ The concentrations of N-acetyl aspartate (NAA), myo-inositol (mI), choline (Cho) and creatine (Cr) were measured in four regions of interest (ROI); the frontal white matter (FWM), basal ganglia (BG), occipital gray (OG) and parietal white matter (PWM).

Diffusion Tensor Imaging (DTI) was performed during the conventional MRI study, using an echo planar imaging based diffusion weighted pulse sequence with the following parameters: time repetition (TR) = 6000 ms (ms), time echo (TE) = 100 ms, six non-collinear directions with diffusion weighting B value of 1000, and 4 number of excitations (averages). An image acquisition matrix of 128 × 128 was used with a field view of 256 × 256 mm² and 16 slices with a 3.0 mm slice thickness. DTI allowed measuring the Fractional Anisotropy (FA) in the corpus callosum (CC), a region that has been implicated in white matter loss and appears to be sensitive to improvement in gene therapy in Canavan disease (Leone et al, unpublished data).

As part of a conventional MRI, T1 and T2 weighted spin-echo images were obtained. Studies were conducted with a Siemens Magnetom Sonata 1.5 Tesla MRI system. T1 weighted spin-echo sequences were acquired with TR/TE = 700/14 ms as described previously.^{12,14} The T1 relaxation times in selected brain regions (anterior and posterior CC and FWM) were measured before and after treatment for the purposes of statistical analysis.

The clinical assessments included gross Motor Function testing (GMFM) at baseline and at 60 days follow-up. All patients underwent laboratory testing of basic metabolic panel and lithium level on bi-monthly basis. Thyroid and liver function panels were also performed monthly to ensure safety.

3. Result

Lithium was well tolerated in this pediatric cohort with no adverse effects. The lithium levels were in the therapeutic range without any clinical or laboratory evidence for toxicity. Mild hyperchloremic metabolic acidosis, commonly seen in Canavan disease, was documented in all patients prior to the study and remained unchanged. There were no renal or liver function abnormalities.

Using the metabolite concentrations measured on H-MRS, the ratios to Cr were calculated and compared at baseline and after sixty days of therapy. To establish the therapeutic effect of lithium in lowering NAA level, the results were subjected to statistical analysis using a non-parametric system (Wilcoxon signed-rank test) which was selected due to the small sample size. The intervention resulted in a modest drop in the NAA/Cr

Table 1 – Summary of Canavan cases.

Number of Cases	Average age in months	Head circumference, percentile	U. NAA	DD or DR	Seizure	ASPA mutations
6	9.5	>95 Percentile	high	present	2 cases	3 cases: compound heterozygote 3 cases: homozygote

U.NAA: urine N-acetyl aspartate. DD: delayed development. DR: developmental regression. ASPA: aspartoacylase.

ratios in all ROI's which was statistically significant in the BG ($p < 0.02$). Table 2 summarizes the mean of the metabolite ratios in the study cohort before and after treatment. Fig. 1 is an example of the MRS images demonstrating a drop in the NAA at the level of the BG post treatment.

The GMFM scores were compared at baseline and after sixty days of treatment. The mean and standard deviation were 5.08 and 2.02 before treatment and 6.13 and 1.75 after treatment which did not show any statistically significant improvement ($p < 0.21$, dependent t test). Clinical improvement was reported in alertness and visual tracking which are not components of GMFM.

Fig. 2 shows the DTI images acquired in two of the cases across the CC before and after treatment. The findings are suggestive of micro-structural improvement in the CC.

The T1 values obtained on selected brain areas (anterior and posterior CC and FWM) were subjected to statistical analysis. A modest drop in the T1 values was noted in the FWM (paired t test, $p = 0.45$).

4. Discussion

We have established the safety and tolerability of lithium in a group of young children with Canavan disease. The NAA/Cr ratios were reduced in all four ROI after treatment with statistical significance in the BG. In the single case previously reported, there was a drop in NAA in all ROI's, though statistical significance could not be established due to extremely small sample size.

NAA is continuously removed by the glial cells where it is hydrolyzed by ASPA to acetate and aspartate. Presence of free acetate is crucial for effective myelin synthesis. Impaired function of ASPA in Canavan disease leads to deficiency of acetate which further impairs myelin formation.⁵

T1 relaxation times in selected brain regions were compared before and after treatment and demonstrated a modest drop in the FWM. The most important factors influencing the T1 values in the developing brain include myelination and water content. Therefore, the T1 values are useful as a surrogate marker for myelin development in children and are expected to drop as myelination takes place.^{15,16} This observation suggests a tendency toward normal brain development with lithium treatment compared to the natural history of the disease.³

The GMFM data failed to demonstrate a significant improvement in patients' motor abilities. GMFM has been utilized for the gene therapy trial of Canavan disease¹⁴ and the

scores were found to correlate with decrease in NAA levels. Although GMFM is less sensitive than the Mullen Test of Early Learning or the Pediatric Evaluation of Disability Index, it was decided to administer the GMFM in this study to power the analysis for planned future clinical trials.

Markedly elevated NAA is unique to Canavan disease and is presumed to have a central role in its pathophysiology. Several papers have addressed the toxic effects of NAA and N-acetyl aspartate glutamate (NAAG). Cultured mouse spinal cord neurons exposed to high doses of NAAG develop excitotoxicity via selective activation of the N methyl D aspartic acid (NMDA) receptors.¹⁷ Similarly, NAA causes an elevation in the intracellular free calcium in a dose dependent fashion which is linked to NMDA activation.¹⁸ Elevated levels of NAA in the tremor rat, which harbors a natural deletion in the ASPA gene, has been shown to induce neuronal excitation caused by activation of glutamate receptor (mGluR).¹⁹ Pliss et al.²⁰ have documented neuronal death in the rat hippocampus following intra-ventricular injection of NAAG. This effect was mediated by activation of NMDA and mGluR and was dampened by co-administration of glutamate receptor antagonists. Wide spread hippocampal neuronal death was observed in four days using modest concentration of NAAG at 0.25 μmol (μM). Others have used intra-ventricular injections of NAA in rats to induce epileptiform discharges in the neurons as well as clinical seizures.^{4,21} The epileptogenic effects of NAAG have been confirmed by stereotactic infusion of the substance into the hippocampus.²² Furthermore, a recent study by Pederzoli et al. has suggested a novel pathway for toxic effects of NAA via impairing the anti-oxidant defenses of the cerebral cortex.⁶

On the other hand, Tranberg et al. have studied the effects of elevated NAA levels for 3 days on cultured slices of rat hippocampus and reported no elevation of NMDA mediated toxicity.²³ The results are contrary to the above citations, possibly due to methodological issues.

The efflux of NAA from the neurons to the ECF is naturally associated with efflux of water molecules. As such, NAA has an important osmo-regulatory function in the brain.²⁴ In Canavan disease, accumulation of NAA impairs the osmotic balance, resulting in elevation of the hydrostatic pressure in the brain and spongiform encephalopathy.²⁵ This is yet another evidence for deleterious consequences of NAA elevation and another reason for targeting NAA for therapeutic purposes.

Our study did not evaluate the effects of lithium on the urinary excretion of NAA. Knowing that H-MRS is not widely available, urinary NAA levels may be an alternative method

Table 2 – The mean of the metabolic ratios to creatine for 6 patients in 4 regions at baseline and follow-up obtained using H-MRS.

Region		NAA		ml		Cho	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
fwm	ratio/Cr	3.04	2.73	0.47	0.34	0.35	0.30
pwm	ratio/Cr	3.52	3.12	0.61	0.57	0.28	0.28
ogm	ratio/Cr	2.86	2.70	0.14	0.17	0.16	0.16
bg	ratio/Cr	2.52	2.20	0.12	0.10	0.21	0.18

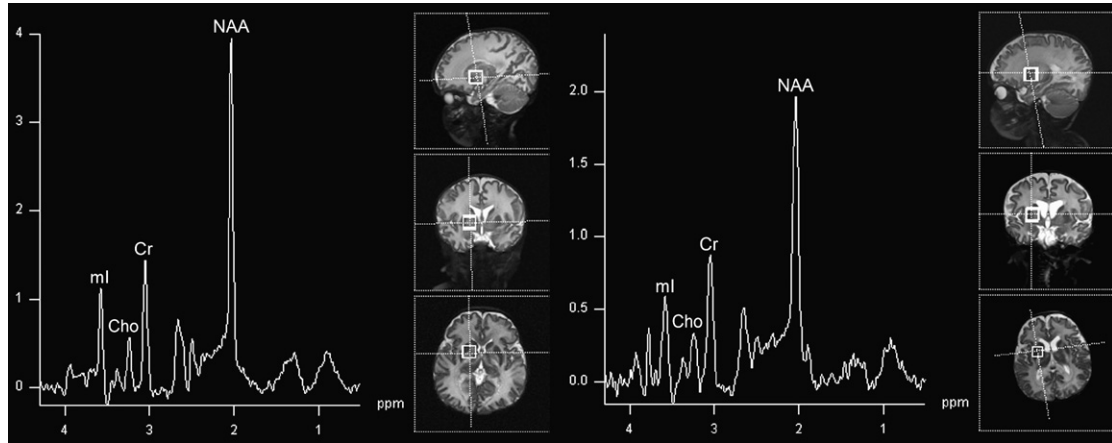


Fig. 1 – Sample MRS on one of the patients: the acquired spectra at the level of the BG show a reduction in the NAA peak after treatment. Before treatment: left, after treatment: right.

for monitoring these patients during lithium therapy. On the other hand, the urinary levels of NAA are quite variable and depend on many factors including dietary changes.

Lithium exerts some of its therapeutic effects by antagonizing glutamate induced excitotoxicity at several levels.²⁶⁻²⁸ Also, lithium is known to offer anti-apoptotic effects by reducing the expression of multiple apoptotic proteins; p53, Bcl-2 associated X protein (Bax) and caspase^{29,30} and by increasing the levels of Brain Derived Neurotrophic Factor (BDNF) and B cell lymphoma 2 (Bcl-2).³¹ The anti-apoptotic and anti-glutamnergic effects of lithium are shown to be present after only one week of exposure.

There are limited number of studies conducted in normal volunteers and patients with bipolar disorder exposed to lithium with contradictory results. The earlier studies had suggested a slight elevation in the NAA level following

exposure to lithium. This was attributed to the neuro-protective and anti-apoptotic properties^{32,33} of the drug. Volumetric brain imaging in normal individuals suggested an increase in the pre-frontal gray matter.³⁴ A more recent study demonstrated no alteration in the NAA level in normal individuals following lithium exposure³⁵ The effects of lithium in Canavan disease seem to be unique and possibly influenced by markedly elevated NAA in the ECF.

Normally, NAA is present at a high concentration of 10–14 mmol in the neurons. In the ECF, the NAA concentration is 80–100 μ M which establishes a large outward transport gradient. At the physiological pH, NAA is a dicarboxylate anion and therefore, cannot be transported by passive diffusion. The oligodendroglial cells are equipped with an active transport system for removing the NAA molecules from the ECF. This is known as sodium coupled carboxylate transporter

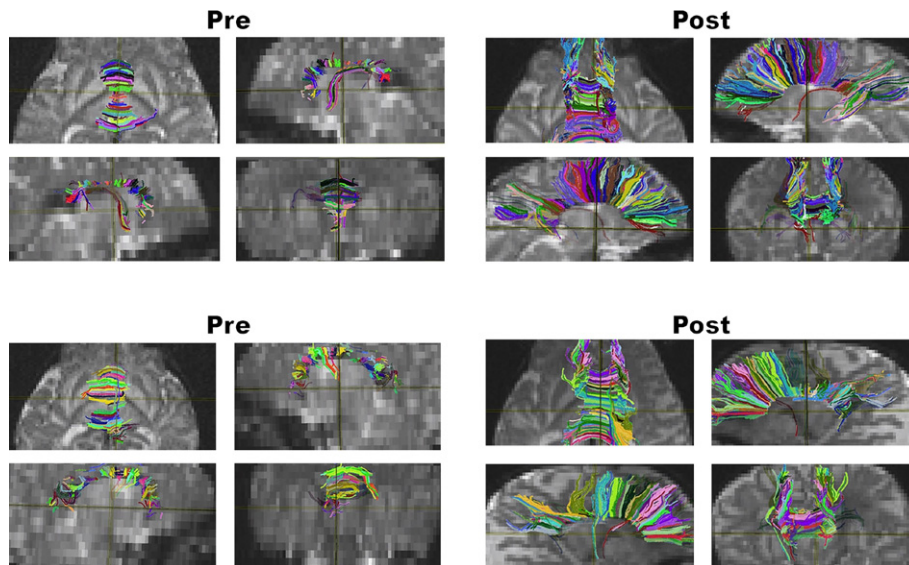


Fig. 2 – Diffusion Tensor Imaging obtained on the brain MRI on two of the patients before and after therapy. An increase in the white matter density at the level of corpus callosum is shown

(NaC) which is present in three forms; NaC1, NaC2 and NaC3.^{36,37} While NaC1 demonstrates low affinity for succinate and other dicarboxylates, NaC3 is a high affinity transporter. NaC2 has a preference for tricarboxylates such as citrate. Lithium strongly inhibits succinate transport through the NaC3 by occupying one of the three Na binding sites.^{36,38} The process of succinate transport by NaC3 is also influenced by sodium concentration and the pH.³⁹ How this attribute of lithium contributes to its therapeutic effect remains to be elucidated.

Based on these results, the future trials should incorporate at least 20 patients to show a statistically significant effect with a power > 0.8 and adequate sensitivity and specificity.

5. Conclusions

Lithium was safe and well tolerated in this cohort of young children with Canavan disease. The cellular and molecular effects of Lithium are diverse and may mitigate the disease process at multiple levels. The key therapeutic effect of lithium seems to be a modest drop in NAA levels, which is an important therapeutic target in Canavan disease. Lithium also modulates the cell signaling pathways to provide protection against apoptosis and NMDA toxicity.

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