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Clinical Observations

Treatment of Creatine Transporter (SLC6A8) Deficiency With Oral S-Adenosyl Methionine as Adjunct to L-arginine, Glycine, and Creatine Supplements

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ABSTRACT

BACKGROUND: Creatine transporter (SLC6A8) deficiency is an X-linked inborn error of metabolism characterized by cerebral creatine deficiency, behavioral problems, seizures, hypotonia, and intellectual developmental disability. A third of patients are amenable to treatment with high-dose oral creatine, glycine, and L-arginine supplementation. **METHODS:** Given the limited treatment response, we initiated an open-label observational study to evaluate the effect of adjunct S-adenosyl methionine to further enhance intracerebral creatine synthesis. **RESULTS:** Significant and reproducible issues with sleep and behavior were noted in both male patients on a dose of 50/mg/kg. One of the two patients stopped S-adenosyl methionine and did not come for any follow-up. A safe and tolerable dose (17 mg/kg/day) was identified in the other patient. On magnetic resonance spectroscopy, this 8-year-old male did not show an increase in intracerebral creatine. However, significant improvement in speech/language skills, muscle mass were observed as well as in personal outcomes as defined by the family in activities related to communication and decision making. **DISCUSSION:** Further research is needed to assess the potential of S-adenosyl methionine as an adjunctive therapy for creatine transporter deficiency patients and to define the optimal dose. Our study also illustrates the importance of pathophysiology-based treatment, individualized outcome assessment, and patient/family participation in rare diseases research.

Keywords: Cerebral creatine deficiency, therapy, personalized medicine, behavior, MR spectroscopy, speech, global developmental delay, seizures

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What this paper adds (2 bullet points):

- Oral adjunct treatment of S-adenosyl methionine at higher dosages causes sleep and behavioral disturbances.
- At lower dosages, it is tolerated with positive effect on speech, language, communications skills, and muscle mass as well as important personal outcomes related to communication, decision-making, and social skills as defined by the family.

Introduction

Creatine transporter deficiency (CTD; MIM 300036) is a cerebral creatine deficiency disorder resulting from genetic alterations of the X-linked *SLC6A8*, which encodes CT1 creatine transporter.[1] The clinical characteristics include intellectual developmental disability, speech delay, autism, seizures, and muscle hypotonia and hypotrophy.[2] The reported prevalence of CTD is 0.4%-1.4% in males with intellectual developmental disability and 2% of X-linked intellectual developmental disability.[3] Urine creatine/creatinine ratio is a biochemical diagnostic marker for CTD in males.[4]

Treatment strategies are based on the hypothesis that correction of intracellular cerebral creatine deficiency will improve clinical outcomes.[5] Creatine supplementation is administered as a monotherapy or in combination with creatine precursors L-arginine and glycine (triple therapy) to overcome creatine deficit through endogenous synthesis within the brain.[6] Our recent systematic literature review identified 10 patients (36%) who responded to treatment, manifested by an increase in cerebral creatine and/or improved clinical parameters. Recognizing the review's limitations, we concluded that a portion of CTD patients is amenable to treatment and recommended systematic screening of intellectual developmental disability patients for CTD to allow for timely treatment with at minimum oral creatine monotherapy (useful in cases with residual transporter function) plus consideration of L-arginine and glycine.[6]

Acknowledging that the majority of CTD patients do not respond to treatment and that outcomes for those are far from optimal, we aimed to further improve treatment. Based on CTD pathophysiology, we hypothesized that addition of S-adenosyl methionine (SAM), a precursor in the creatine synthesis pathway, to the oral triple therapy might further enhance cerebral endogenous synthesis and may result in observable biochemical, physical, neurodevelopmental, and biochemical changes. SAM is an important methyl donor in the synthesis of creatine from guanidinoacetate and can cross the blood–brain barrier intact. SAM administered in high pharmacological doses has been reported in the treatment of depression, neurologic disorders, osteoarthritis, and hepatic problems with low incidence of side effects and long-term sustained tolerance.[7]

Building on these experiences, we initiated an open-label trial to evaluate the safety and efficacy of oral supplementation of SAM in improving cerebral creatine levels and clinical outcomes in two patients with CTD as an adjunct to creatine monohydrate, 400 mg/kg/day; L-arginine, 400 mg/kg/day; and L-glycine, 250 mg/kg/day divided into three doses. These two patients were previously reported in our systematic review for their outcomes on triple therapy.[6] The protocol was exempted from review by the Children's and Women's Research Ethics Board, University of British Columbia. However, the Innovative Treatment Intervention Protocol Committee in the Department of Pediatrics, University of British Columbia, approved it. Parents of both patients provided informed consent for publication.

Patient Descriptions

For patient 1 and insofar as available for patient 2, primary and secondary outcomes as well as safety parameters are listed in the Table.

Patient 1 is now an almost 8-year-old boy who presented with mild intellectual developmental disability, autism, generalized hypotonia, clumsiness, and complex partial seizures well-controlled by a single antiepileptic. He was diagnosed at age 4.9 years with CTD based on an abnormal urinary screen, decreased intracerebral creatine on proton magnetic resonance spectroscopy (H-MRS) and molecular analysis (*SLC6A8* c.859delC); he is described in the 2014 review by Dunbar et al. (patient 1).[6] After 22 months on treatment with triple therapy, SAM was started at 50 mg/kg/day, taken as 400 mg in the morning, 400 mg in the afternoon, and 300 mg in the night ingested simultaneously with creatine, glycine, and L-arginine supplements. Initially, there were reports of improved speech; however, treatment was discontinued after 3 months because of simultaneous reports of significant sleep disruptions resulting in daytime exhaustion as well as significant behavior issues that included screaming, tantrums, throwing things, and biting. Both symptoms related to behavior and sleep disappeared within 2-3 days without SAM. After 6 weeks without symptoms, SAM was reinitiated at 200 mg three times daily (50% of study treatment dose 25 mg/kg/day); the same albeit milder symptoms appeared after 2-3 days of treatment. The dose was further reduced to 200 mg twice daily (17 mg/kg/day); this dose has been well-tolerated without any side effects. *A priori* treatment expectations are measured using the Personal Outcomes of Specific Interest Technique; more information as well as a patient narrative is provided in Supplement 1. Patient's outcomes on treatment are listed in Table 1.

Patient 2 is now an 9-year old boy who presented with moderate intellectual developmental disability and significant hypotonia; he was diagnosed at age 4.7 years with CTD based on an abnormal urinary screen, decreased intracerebral creatine on H-MRS and molecular analysis (*SLC6A8* c. c.1222+1224del TTC). He is well-described in the 2014 review by Dunbar et al.[6] After 4 years on triple therapy, he was started on oral SAM 50 mg/kg/day. After 2 weeks of treatment, he also developed major behavioral issues, which included restlessness, anxiety, hyperactivity, and episodes of screaming and excessive talking. At the same time, his quality of speech and communication had improved ("he was able to put words together and say actual phrases"); see the Table. Given the significant side effects, SAM was discontinued after a few weeks, and the family withdrew from the study. They did continue him on the triple therapy according to email communications, but did not come for any follow-up assessment.

Discussion

This is the first report of the safety and efficacy of oral supplementation of SAM as adjunct treatment for CTD. Given the high frequency of CTD, it is important to establish an adequate intervention. Our review showed that 36% of the patients do respond to currently available treatment options either by an increase in intracerebral creatine and/or clinical parameters (evidence level IV).[6] Treatment response rate and effect size are inadequate, however. So while awaiting true breakthroughs such as with cyclo-

TABLE.
Treatment Outcomes

	Patient 1	Patient 2
Age at start SAM	7 years	8 years
Dosage SAM (time interval)*	50 mg/kg/day (3.3 months) 25 mg/kg/day (2 weeks) 17 mg/kg/day (8 months)	50 mg/kg/day (4 weeks)
Safety†	No safety concerns	No safety concerns
Adverse effects‡	Sleep disruptions, screaming, tantrums, throwing objects, and biting (disappeared on lower dosage)	Hyperactivity, restlessness, anxiety, screaming, and excessive talking
Creatine change basal ganglia§	No change: stable when compared with pre-SAM MRS	Not done
Creatine change white matter‖	No change: stable when compared to pre-SAM MRS	Not done
Neurodevelopmental testing¶	Improvement in overall understanding and production of language and language-based reasoning, along with other developmental gains linked to his increased ability to interact with others using language	Not done
Behavior#	More alert and responsive	No follow-up
Seizure control**	No change: seizures remained well-controlled, electroencephalograph remained normal	No seizures
Muscle mass††	10% increase in fat-free mass, above 50th percentile for both height and weight for age (World Health Organization growth chart for Canada)	No follow-up
Family expectations‡‡	Communication (+2) Decision-making (+1) Social skills (−2)	No follow-up

Abbreviations:
ALT = Alanine transaminase
AST = Aspartate transaminase
BUN = Blood urea nitrogen
GFR = Glomerular filtration rate
GGT = Gamma-glutamyltransferase
H-MRS = Proton magnetic resonance spectroscopy
MRS = Magnetic resonance spectroscopy
SAM = S-adenosyl methionine

* Dosage in mg/kg/day divided in three doses (time interval during which SAM was administered).
† Safety (liver, kidney, nutrition) was monitored with the following analyses in blood and urine: AST, ALT, GGT, BUN, creatinine, albumin, electrolytes, GFR, tubular reabsorption fraction, fasting plasma total homocysteine and amino acids (arginine, glycine, methionine), plasma guanidinoacetate and creatine, urine (crystals).
‡ Side effects as reported by the families.
§ Measurement of the creatine content in basal ganglia using cranial H-MRS.
‖ Measurement of the creatine content in basal ganglia using cranial H-MRS.
¶ Neurodevelopmental outcome assessed using standardized psychometric scales (Wechsler Intelligence Scale for Children, 4th edition; Bracken Basic Concept Scale, 3rd edition; Peabody Picture Vocabulary Test, 4th edition; Expressive Vocabulary Test, 2nd edition; Beery-Buktenica Developmental Test of Visual Motor Integration, 6th edition; Behaviour Rating Inventory of Executive Function, Adaptive Behaviour Assessment System, 2nd edition; ADHD Rating Scale IV; Behaviour Assessment System for Children −2 pre and 14 months posttreatment, which includes a 6-week period during which SAM was stopped.
# Behavioral changes noted.
** Seizure activity measured using seizure logbook and electroencephalograph.
†† Muscle mass analysis by measuring the body composition using Bioelectrical Impedance Analyzer.
‡‡ The Personal Outcomes of Specific Interest Technique tool was used to ascertain the family's a priori expectations; + indicates these was met.

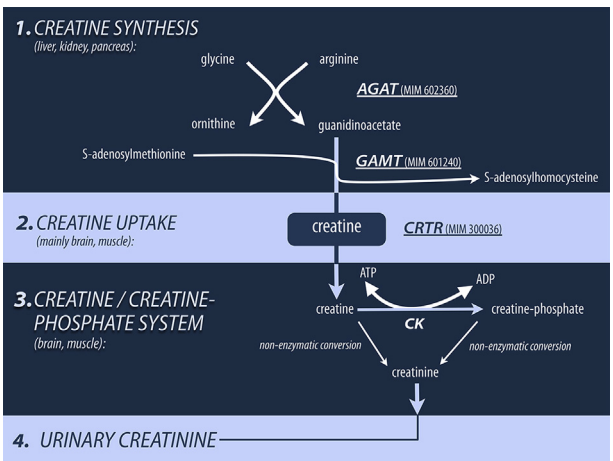

FIGURE.
Creatine synthesis and metabolism.[6] (The color version of this figure is available in the online edition.)

creatine,[8,9] we aimed to enhance current treatment choices through SAM.

There was no significant change in primary outcome—cerebral creatine measured using H-MRS in patient 1. This is not surprising as only a proportion (25% of 28 patients in the review by Dunbar et al.) of patients reported in the literature showed an increase in intracerebral creatine using different treatment combinations.[6] Definite changes, however, occurred in the secondary outcomes for patient 1. Most importantly, formal neurodevelopmental testing showed a significant improvement in language and communication skills. Additionally, improvement was reported in a priori treatment expectations evaluated with the Personal Outcomes of Specific Interest Technique, (an in-house tool) in the domain of communication and decision-making. Furthermore, an increase in his muscle mass was noted. This is an indirect confirmation of the principle of enhancement of creatine synthesis through SAM. Seizures remained well controlled for patient 1

during SAM treatment with a pre- and posttreatment electroencephalograph.

Both patients showed significant behavioral disturbances, restlessness, and sleep problems on a SAM dose of 50 mg/kg/day. A safe and tolerable dose was established in patient 1 after series of dechallenge and rechallenge with SAM. These important adverse reactions were not expected considering the good safety profile of SAM; it is the reason why we started the therapy at a dose of 50 mg/kg/day. Based on our experience, we recommend starting at a dose of 20 mg/kg/day or lower, divided into two or three doses, and avoid administration in the evenings given the potential for sleep disruption. Because SAM is an enteric-coated tablet, it should be taken whole to achieve peak plasma concentrations; however, this can be challenging in CTD patients given their age and developmental ability.

The limitations of this study should be acknowledged. In addition to SAM, patient 1 was already on triple therapy for 22 months, with multiple other ongoing (albeit unchanged during SAM) interventions such as physiotherapy and speech therapy. We recognize multifactor contributions to developmental progress. Furthermore, this study was open label and the reporting of outcomes therefore subject to bias. Sensitivity of measures applied to evaluate the outcomes is also an area of concern. The H-MRS method used for cerebral creatine measurement is a semiquantitative method. The psychometric scales for evaluation of neurodevelopmental outcome have not been normed for this rare disease population. The absence of norms creates challenge for detecting statistically significant differences that reflect clinical and meaningful differences that families observe and associate with different treatment protocols. We have tried to overcome this limitation by using the Personal Outcomes of Specific Interest Technique tool to capture families' expectations *a priori*; this individualized approach is important in assessing treatment outcomes of rare diseases such as these. According to the Centre for Evidence-based Medicine criteria (www.cebm.net), the evidence level for the effects of this treatment is IV.

Despite these limitations, this study adds important information to the literature on treatment of CTD patients: As adjunct to supplementation with creatine, L-glycine, and L-arginine, oral SAM has potential adverse effects at higher dosages, which include behavioral and sleep disruptions. At a dosage of 20 mg/kg/day, it was safe and tolerated in patient 1, with the potential to further improve muscle mass and speech/communication. More research is needed to replicate these findings in larger number of patients, to further delineate the optimal and safe dosage, and to generate more evidence for the effects on primary and secondary outcomes, and the timing of treatment initiation. Finally, this study illustrates the importance of individualized medicine in rare diseases for the definition of relevant outcomes; active participation of patients and families enhances studies challenged by small patient numbers. This approach will also prove useful for future studies evaluating the effectiveness of interventions currently in development phase, which use chemically modified creatine molecules (e.g., cyclocreatine[8]) and coupling of creatine to molecules that have their own carrier.[9]

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References

1. Salomons G, van Dooren S, Verhoeven N, et al. X-Linked Creatine-Transporter Gene (SLC6A8) Defect: A New Creatine-Deficiency Syndrome. *Am J Hum Genet*. 2001;68:1497-1500.
2. van de Kamp J, Betsalel O, Mercimek-Mahmutoglu S, et al. Phenotype and genotype in 101 males with X-linked creatine transporter deficiency. *J Med Genet*. 2013;50:463-472.
3. van de Kamp J, Mancini G, Salomons G. X-linked creatine transporter deficiency: clinical aspects and pathophysiology. *J Inherit Metab Dis*. 2014;37:715-733.
4. Almeida L, Verhoeven N, Roos B, et al. Creatine and guanidinoacetate: diagnostic markers for inborn errors in creatine biosynthesis and transport. *Mol Genet Metab*. 2004;82:214-219.
5. Stockler S, Schutz P, Salomons G. Cerebral creatine deficiency syndromes: clinical aspects, treatment and pathophysiology. *Subcell Biochem*. 2007;46:149-166.
6. Dunbar M, Jaggumantri S, Sargent M, Stockler-Ipsiroglu S, van Karnebeek C. Treatment of X-linked creatine transporter (SLC6A8) deficiency: systematic review of the literature and three new cases. *Mol Genet Metab*. 2014;112:259-274.
7. Bottiglieri T. S-Adenosyl-L-methionine (SAMe): from the bench to the bedside—molecular basis of a pleiotrophic molecule. *Am J Clin Nutr*. 2002;76:1151S-1157S.
8. Kurosawa Y, DeGrauw T, Lindquist D, et al. Cyclocreatine treatment improves cognition in mice with creatine transporter deficiency. *J Clin Invest*. 2012;122:2837-2846.
9. Trotier-Faurion A, Passirani C, Béjaud J, et al. Dodecyl creatine ester and lipid nanocapsule: a double strategy for the treatment of creatine transporter deficiency. *Nanomedicine (Lond)*. 2015;10:185-191.

Appendix. Supplement

A priori treatment expectations are measured using the Personal Outcomes of Specific Interest Technique (POSI) technique. It is a personalized patient reported outcome tool developed in house at BC Children's Hospital. This technique integrates the methods of therapeutic emplottment and Goal Attainment Scaling[1,2] to identify outcomes that patients consider as being important in their daily life and expect the treatment to have an effect.

Patient 1:

PSOI - 01	Communication/Conversation
Levels of predicted attainments	
Much less than the expected level of outcome (−2)	Not being able to communicate and reply back in a full sentence even once a day
Somewhat less than the expected level of outcome (−1)	Being able to communicate and reply back in full sentences at least 1-2 times in a week
Expected level of outcome (0)	Being able to communicate and reply back in full sentences at least 1-2 times every day
Somewhat more than the expected level of outcome (+1)	Being able to communicate and reply back in full sentences more than 3-5 times every day
Much more than the expected level of outcome (+2)	Being able to communicate and reply back in full sentences more than 6-10 times every day
PSOI - 02	**Decision-Making**
Levels of predicted attainments	
Much less than the expected level of outcome (−2)	Not being able to make clear decisions and choose what he wants without influence even once a day
Somewhat less than the expected level of outcome (−1)	Being able to make clear decisions and choose what he wants without influence at least 1-2 times in a week
Expected level of outcome (0)	Being able to make clear decision and choose what he wants without influence at least 3-5 times everyday
Somewhat more than the expected level of outcome (+1)	Being able to make clear decision and choose what he wants without influence more than 6-10 times everyday
Much more than the expected level of outcome (+2)	Being able to make clear decision and choose what he wants without influence more than 11 -15 times everyday
PSOI - 03	**Social Skills**
Levels of predicted attainments	
Much less than the expected level of outcome (−2)	Not able to spend time with kids in his class at school play time between 12:30 and 2:00 pm even at least once in a week
Somewhat less than the expected level of outcome (−1)	Not able to spend time with normal grade kids at school play time between 12:30 and 2:00 pm even once in a week
Expected level of outcome (0)	Spending time with normal grade kids at school play time between 12:30 and 2:00 pm at least 1 time in a week
Somewhat more than the expected level of outcome (+1)	Spending time with normal grade kids at school play time between 12:30 and 2:00 pm 2 times in a week
Much more than the expected level of outcome (+2)	Spending time with normal grade kids at school play time between 12:30 and 2:00 pm 3 times in a week

Parent narrative for patient 1:

"Although science may not yet have developed the tools to measure the changes associated with the treatment, nature has. Nobody knows a child like a mother and I believe my son's journey from head banging and stumbling to a boy that many don't realize is challenged, has undoubtedly been enhanced by his supplements. Whilst he may wear his pants backwards occasionally, my son is now able count to ten!".

References

1. Mattingly C. The concept of therapeutic emplottment. *Soc Sci Med*. 1994;38:811-822.
2. Kiresuk T, Lund S, Larsen N. Measurement of goal attainment in clinical and health care programs. *Drug Intell Clin Pharm*. 1982;16: 145-153.