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Safety, efficacy and physiological actions of a lysine-free, arginine-rich formula to treat glutaryl-CoA dehydrogenase deficiency: Focus on cerebral amino acid influx

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ABSTRACT

Striatal degeneration from glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type 1, GA1) is associated with cerebral formation and entrapment of glutaryl-CoA and its derivatives that depend on cerebral lysine influx. In 2006 we designed a lysine-free study formula enriched with arginine to selectively block lysine transport across cerebral endothelia and thereby limit glutaryl-CoA production by brain. Between 2006 and present, we treated twelve consecutive children with study formula (LYSx group) while holding all other treatment practices constant. Clinical and biochemical outcomes were compared to 25 GA1 patients (PROx group) treated between 1995 and 2005 with natural protein restriction (dietary lysine/arginine ratio of 1.7 ± 0.3 mg:mg). We used published kinetic parameters of the y + and LAT1 blood–brain barrier transporters to model the influx of amino acids into the brain. Arginine fortification to achieve a mean dietary lysine/-arginine ratio of 0.7 ± 0.2 mg:mg was neuroprotective. All 12 LYSx patients are physically and neurologically healthy after 28 aggregate patient-years of follow up (current ages 28 ± 21 months) and there were no adverse events related to formula use. This represents a 36% reduction of neurological risk (95% confidence interval 14–52%, $p = 0.018$) that we can directly attribute to altered amino acid intake. During the first year of life, 20% lower lysine intake and two-fold higher arginine intake by LYSx patients were associated with 50% lower plasma lysine, 3-fold lower plasma lysine/arginine concentration ratio, 42% lower mean calculated cerebral lysine influx, 54% higher calculated cerebral arginine influx, 15–26% higher calculated cerebral influx of several anaplerotic precursors (isoleucine, threonine, methionine, and leucine), 50% less 3-hydroxyglutarate excretion, and a 3-fold lower hospitalization rate (0.8 *versus* 2.3 hospitalizations per patient per year). The relationship between arginine fortification and plasma lysine indicates that transport competition exists at both cerebrovascular and gastrointestinal barriers, suggesting their co-administration is key to efficacy. Monitoring the ratio between lysine and arginine in diet and plasma may prove a useful strategy for treating children with GA1.

1. Introduction

Children with glutaryl-CoA dehydrogenase deficiency (GCDH; glutaric aciduria type 1, GA1) can suffer selective striatal degeneration that presents as insidious motor delay or acute motor regression during infancy [1–3]. Striatal lesions cause movement disorders that are notoriously difficult to treat. Generalized dystonia results in chronic complications such as pain, dysphagia, aspiration, laryngospasm, scoliosis, and restrictive lung disease and often culminates in untimely death [4].

When the Clinic for Special Children was founded in 1989, management of GA1 focused on preemptive diagnosis, natural protein restriction, sustained enteral carbohydrate during illnesses, and an inpatient protocol designed to suppress protein catabolism and promote renal clearance of organic acids. Newborn screening for GA1 began statewide in 1994 and we started consistently treating with L-carnitine in 1995. Collectively, these measures reduced brain

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injury from 94% to 36% among Amish GA1 patients. However, this figure remained unchanged during the use of prophylactic anticonvulsants and vitamin cofactors (including riboflavin, pantothenate, and various antioxidants) in the decade that followed [4]; these therapies conferred no neuroprotective advantage and so were abandoned [2].

Although population-based studies established the value of newborn screening for GA1 [3–7], there remained uncertainties about optimal treatment of asymptomatic infants [8]. Outcome data from multiple centers were evidence for the value of dietary lysine restriction [3,9], but excellent outcomes could be achieved without such restriction [10]. Moreover, because dietary lysine restriction and L-carnitine were almost always used in combination, it was impossible to discern their relative importance. Whatever treatment was adopted at a particular center, investigators agreed that traditional chemical markers did not produce an accurate picture of metabolic control or neurological risk [3,4,8,11] while mounting evidence bolstered the concept of GA1 as a *cerebral* organic aciduria [11–15]; *i.e.* that GCDH deficiency leads to chemical derangements *within the brain* that underlie the risk for neuronal injury but are not reflected by measurements from urine or blood.

A 1988 study of healthy fruit bats (*Rousettus aegypticus*) gave the first indication that systemic and cerebral glutarate metabolism are compartmentalized [16]. These animals have naturally low levels of GCDH in liver and excrete glutaric acid levels comparable to GCDH-deficient humans, but they have abundant GCDH activity in brain which prevents cerebral accumulation of glutaryl-CoA and presumably protects the nervous system. In contrast, humans with GA1 express mutant GCDH transcript in brain, where glutarate accumulates to concentrations of 500–2000 μmol/kg wet weight, two to three orders of magnitude higher than plasma or cerebrospinal fluid [11]. Based on these data, in 2005 we first posited that GCDH-deficient human brain produced the bulk of glutaryl-CoA from imported lysine [17]. An intercompartmental model of lysine flux suggested that the growing human brain could produce as much as 5000 to 12,000 μmoles of glutaryl-CoA per kg of tissue from lysine each day. Subsequent animal experiments verified the idea that glutaryl-CoA and its derivatives, once produced within the brain, become trapped due to inadequate cerebral systems for dicarboxylic acid export and low capacity to form carnitine and glycine conjugates [13,14,18–23].

Based on this line of reasoning, in 2006 we designed a study formula to selectively restrict *cerebral* lysine uptake by exploiting competition between lysine and arginine at the blood–brain barrier (Fig. 1) [24,25]. These amino acids share a common cerebrovascular cationic transporter (y + system) [26] with estimated *in situ* K_m values of 70 μM (lysine), 56 μM (arginine), and 109 μM (ornithine)[27]. Thus arginine, within physiological meaningful plasma concentration ranges (reference range 90 ± 32 μM), can modulate cerebral lysine uptake. Other lysine-free medical foods rich in arginine (85–103 mg/g protein, Abbott Nutrition, SHS International) already in use [9,28,29] have never been subjected to a detailed analysis of their effects on amino acid physiology or brain nutrition. Here we examine clinical and biochemical effects of a new formula that couples lysine-restriction to selective fortification of arginine and a group of several essential amino acids. Based on our calculations, this strategy can in principle reduce brain lysine uptake by 50% while increasing the cerebral uptake of arginine and other key anaplerotic substrates. Twelve consecutive GA1 patients treated with study formula over 28 aggregate patient-years are healthy and free of brain injury. This treatment provides a therapeutic advantage over protein-restriction among our patient population. Our results strengthen the concept of arginine fortification and suggest that the main therapeutic action of GA1 medical foods is mediated at the level of cerebral amino acid nutrition.

2. Patients and methods

2.1. Study protocol and groups

Between 2006 and 2011, 12 children (6 girls) were diagnosed with GA1 by newborn screening (N = 8) or molecular analysis of

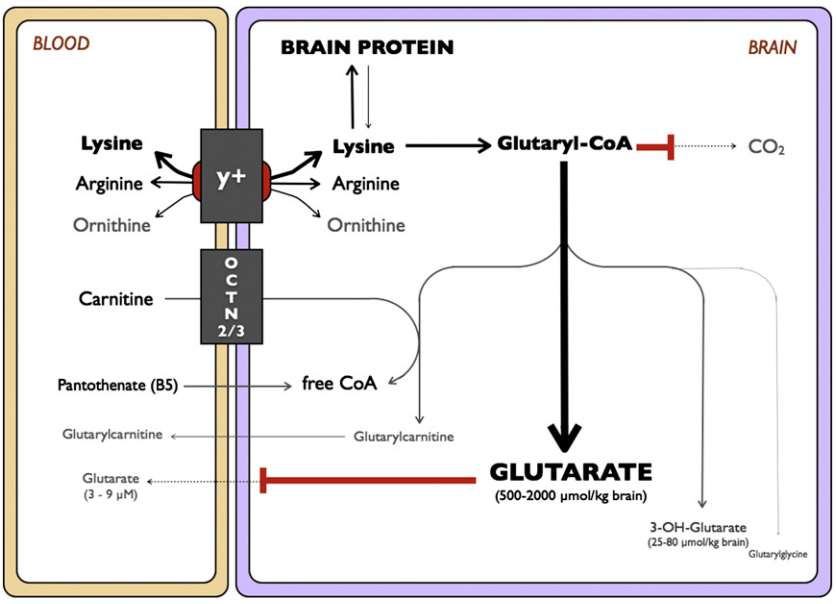

Fig. 1. Proposed scheme of cerebral glutarate loading. Lysine, arginine, and ornithine traverse the blood–brain barrier by a common bidirectional facilitative transporter (y+). Arginine exerts significant competition on lysine transport within the physiologic concentration range. Intracerebral lysine is used for protein accretion or can be channeled to the mitochondrial degradation pathway. In GCDH-deficient individuals, non-degraded glutaryl-CoA is formed in mitochondria proximal to the GCDH block (designated with red line), and is the source of glutarate and 3-hydroxyglutarate. There are no high-affinity cerebrovascular transport systems for these organic acids and so they accumulate to high concentrations in brain tissue (2- to 3-orders of magnitude higher than plasma or cerebrospinal fluid). L-carnitine enters brain via a separate transport system (OCTN2/3) and can be used to a limited extent to form glutarylcarnitine. Glycine conjugation is negligible. The relative quantitative importance of each pathway is designated by line and font weights.