Progress toward Acetate Supplementation Therapy for Canavan Disease: Glyceryl Triacetate Administration Increases Acetate, but Not N-Acetylaspartate, Levels in Brain

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ABSTRACT

Canavan disease (CD) is a fatal genetic neurodegenerative disorder caused by mutations in the gene for aspartoacylase, an enzyme that hydrolyzes N-acetylaspartate (NAA) into L-aspartate and acetate. Because aspartoacylase is localized in oligodendrocytes, and NAA-derived acetate is incorporated into myelin lipids, we hypothesize that an acetate deficiency in oligodendrocytes is responsible for the pathology in CD, and we propose acetate supplementation as a possible therapy. In our preclinical efforts toward this goal, we studied the effectiveness of orally administered glyceryl triacetate (GTA) and calcium acetate for increasing acetate levels in the murine brain. The concentrations of brain acetate and NAA were determined simultaneously after intragastric administration of GTA. We found that the acetate levels in brain were increased in a dose- and time-dependent manner, with a 17-fold increase observed at 1 to 2 h in 20- to 21-day-old mice at a dose of 5.8 g/kg GTA. NAA levels in the brain were not significantly increased under these conditions. Studies using mice at varying stages of development showed that the dose of GTA required to maintain similarly elevated acetate levels in the brain increased with age. Also, GTA was significantly more effective as an acetate source than calcium acetate. Chronic administration of GTA up to 25 days of age did not result in any overt pathology in the mice. Based on these results and the current Food and Drug Administration-approved use of GTA as a food additive, we propose that it is a potential candidate for use in acetate supplementation therapy for CD.

Canavan disease (CD) is a fatal hereditary disorder of the brain that is caused by mutations in the gene for the enzyme aspartoacylase (ASPA) (Kaul et al., 1994). ASPA acts to deacetylate N-acetylaspartate (NAA), thus generating free acetate in the brain. The clinical symptoms of the disease include poor head control, macrocephaly, marked developmental delay, hypotonia, optic atrophy, seizures, and death in early childhood (Adachi et al., 1973). The pathologies associated with CD include cortical and subcortical spongy degeneration, widespread myelin defects, and hypertrophy and hyperplasia of astrocytes (Matalon et al., 1995). Biochemical analyses have shown that hypomyelination is a characteristic feature of CD (Kamoshita et al., 1968). Determination of the elevated levels of urinary NAA is the most reliable diagnostic procedure for CD because CD patients were found to have 10- to 100-fold higher excretion of NAA in the urine than normal (Kvittingen et al., 1986).

CD is prevalent among Ashkenazi Jews with the two predominant mutations of C693A and A854C found in 98% of the Jewish CD patients examined. Mutations in the ASPA gene occur less frequently among non-Jewish patients. The mutations among the non-Jewish patients are different and more diverse; the most common mutation is a C914A substitution, and this allele has been observed in 35.7% of non-Jewish European individuals with Canavan disease (Kaul et al., 1994). Other mutations in non-Jewish patients can randomly reside on exons 1 to 6. Currently, there is no effective treatment for CD, and children with the disorder are treated symptomatically, especially with respect to reducing the incidence and severity of seizures past the age of 3.

NAA is an abundant (5–10 mM) amino acid derivative present in the mammalian nervous system (Tallan et al., 1956) and is found predominantly in neurons (Moffett and Namboodiri, 1995). It is known that the acetyl moiety of NAA is incorporated into fatty acids and lipids associated with...
myelin (D’adamo and Yatsu, 1966; Burri et al., 1991; Mehta and Namboodiri, 1995; Chakraborty et al., 2001) and that the rate of this incorporation is highest during the period of heightened postnatal CNS myelination (D’adamo et al., 1968; Burri et al., 1991). The developmental appearance of NAA and its synthesizing and metabolizing enzymes correlate with the period of active CNS myelination. Autosomal recessive defects in the ASPA gene give rise to NAA accumulation in the nervous system that is associated with brain edema and a progressive loss of oligodendrocytes and myelin (Matalon et al., 1995). These and other observations led to our hypothesis that a deficiency in the supply of NAA-derived acetate results in decreased synthesis of myelin-related fatty acids and lipids, and this lipogenic insufficiency causes CD (Mehta and Namboodiri, 1995; Namboodiri et al., 2000). Recently, we tested this hypothesis by studying acetate levels and the rate of myelin lipid synthesis in the mouse model of CD and showed that acetate levels were reduced, and lipid synthesis was impaired at the stage of peak postnatal CNS myelination (Madhavarao et al., 2005).

Rarely are fatal genetic disorders treatable by means of dietary supplementation, but in the case of Canavan disease, we propose that acetate supplementation during the first several years of life can prevent much of the pathogenesis. In our preclinical studies on the possible use of dietary supplementation therapy for CD, we have examined GTA and calcium acetate as potential exogenous sources for providing acetate to the brain. GTA is a nontoxic glyceryl triester of acetic acid that is widely used as a solvent and plasticizer in perfumery, tanning, and dyes; a food additive; a gelatinizing agent in cosmetics; and it is also used in external medicine. Biochemical studies on GTA have shown that GTA is hydrolyzed in vivo by all tissues of mammals, indicating that it will be readily metabolized even when large doses are administered (Bach and Metais, 1970). Calcium acetate is currently approved as a drug for the treatment of kidney disease to control high blood phosphate levels (Schiiller et al., 1989). However, administration of calcium acetate causes gastrointestinal irritation, making its use for the treatment of CD problematic. In the present study, we used a modified method (Patience and Thomas, 1982) to analyze the concentrations of brain acetate and NAA simultaneously to ascertain if GTA is a good candidate for delivery of acetate to the brain and to ensure that brain NAA levels are not increased by GTA treatment, which could exacerbate CD pathology.

### Materials and Methods

**Materials.** p-Bromophenacyl-8 reagent (0.1 mmol/ml p-bromophenacylbromide and 0.005 mmol/ml crown ether in acetoneitrile) was purchased from Pierce Chemical (Rockford, IL). GTA (fresh weight, 218.21; liquid, density, 1.158), calcium acetate, NAA, sodium acetate, and HPLC-grade acetonitrile were obtained from Sigma-Aldrich (St. Louis, MO). Cation exchange resin (AG 50W-X8) was purchased from Bio-Rad (Hercules, CA). [1-14C]Acetyl-coenzyme A and l-([U-14C]aspartic acid were obtained from Amersham Biosciences Inc. (Piscataway, NJ). Liquid scintillation cocktail was obtained from Beckman Coulter (Fullerton, CA). Deionized and filtered Super Q water (Millipore Corporation, Billerica, MA) was used for all purposes. [14C]Acetate was prepared from [1-14C]acetyl-coenzyme A (Namboodiri and Klein, 1979), and [14C]N-acetylaspartate was prepared from L-[1-14C]aspartic acid as described previously (Madhavarao et al., 2002).

**Animal Studies.** Male C57BL/6 mice of varying stages of development were obtained from Charles River Laboratories, Inc. (Wilmington, MA). The animals were allowed free access to food and water, and newborn mice were kept with their mothers until they were weaned. Three to five animals/group were used in each set of experiments. Different doses (2.9, 4.3, 5.8, and 7.2 g/kg animal weight) GTA and 26.5 mmol/kg animal weight calcium acetate dissolved in water were administered intragastrically to mice at 20 to 21 days old, and the animals were sacrificed 1 h after administration. A group of 20- to 21-day-old mice was given 5.8 g/kg GTA and was sacrificed at different intervals (1, 2, 4, 8, and 12 h). We also analyzed acetate concentrations after GTA feeding in mice at varying stages of development. Mice 7 to 8 and 12 to 14 days old were administered two different doses of GTA (1.45 and 2.9 g/kg) and were sacrificed after 1 h. Mouse 20 to 21 days old and adults were given 2.9 and 5.8 g/kg GTA and were also sacrificed after 1 h. Brains and livers were removed, frozen on dry ice, and stored at −80°C until used. For toxicity studies with chronic GTA administration, starting from the 7th day of age, a group of five animals was fed GTA orally two times a day until 25 days of age. The dose of GTA was 2.9 g/kg animal weight until 15 days of age; thereafter, the dose was increased to 5.8 g/kg animal weight to achieve the maximal increase of acetate level in the brain. All animal procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals.

**Tissue Extraction.** Brain and liver homogenates (20% w/v) were prepared in methanol/water (90:10 volume) by sonication at 0−4°C in an Ultrasonic Homogenizer (4710 Series; Cole-Parmer Instrument Co., Vernon Hills, IL) with the microtip probe limit set at 5 and duty cycle set at 30%. The homogenate was then centrifuged at 10,000 rpm for 30 min. The supernatant was lyophilized, and the residue was dissolved in water and passed through a cation-exchange column (AG 50W-X8). The eluate and two column washings were pooled, adjusted to pH 7 to 8 with 10 mM KOH, and lyophilized again for derivatization.

**Preparation of Phenacyl Esters.** p-Bromophenacyl ester derivatives of acetate and NAA were prepared using p-bromophenacylbromide and crown ether (a phase transfer catalyst) in acetonitrile (Durst et al., 1975; Persson et al., 1991). Briefly, 100 μl of acetonitrile and 40 μl of p-bromophenacylbromide reagent were added to the lyophilized tissue extract prepared as above in a microcentrifuge tube (each vial containing 200-μl equivalent of the 20% tissue homogenate), and the vials were tightly capped. The samples were vortex-mixed and kept in a water bath at 80°C for 1 h with occasional stirring. The vials were then cooled, the solvent removed by lyophilization, and the samples reconstituted in the mobile phase and applied to HPLC.

**HPLC Analysis of Phenacyl Esters of Acetate and NAA.** HPLC analysis was performed isocratically using a Hewlett Packard HPLC system (series 1100). Separation of phenacyl derivatives was carried out on a reverse-phase column (Bio-Advantage 100 C18, 5 μm, 250 × 4.6 mm) with a mobile phase of acetonitrile/water (50:50 by volume) at 25°C. The flow rate was 1.0 ml/min with detection at 250 nm, and the signal was processed by the integrator. The dried phenacyl esters were dissolved in the mobile phase solvent mixture, and 100 μl was injected over the column. The peaks of phenacyl esters of acetate and NAA from the tissues were identified via their retention times in relation to the internal standards sodium acetate and NAA.

For recovery studies, known amounts of radiolabeled acetate and NAA were added to the tissue extract with 90% methanol, and the samples were processed as described above. The HPLC fractions corresponding to phenacyl derivatives of acetate and NAA were collected and pooled separately, and the radioactivity was determined using a Beckman Liquid Scintillation counter system. The percentage of recovery was calculated based on the amount of radioactivity added to the extracts.
Statistical Analysis. All data are presented as means ± S.D. In the dose-response and time-course studies, the data were analyzed for significance by one-way analysis of variance to determine differences between groups, and Dunnett’s post hoc analysis (α = 0.05) was used to compare all the groups against the control. The data analyses were carried out using SPSS 11.0 software (SPSS Inc., Chicago, IL). In all other experiments, statistical analysis was carried out using Student’s t test, and the values were compared with each other. P values of 0.05 or less were considered significant.

Results

The modified assay method used in the present study involved the extraction of the tissue using 90% methanol, purification using cation-exchange column chromatography, derivatization using p-bromophenacylbromide, and separation/estimation using reverse-phase HPLC. By using this method, the levels of acetate and NAA in nanogram quantities were determined simultaneously in the brains of mice at various stages of development following intragastric administration of GTA and calcium acetate. Figure 1 shows representative HPLC chromatograms of phenacyl derivatives of acetate and NAA standards and the corresponding peaks in brain tissue samples. Under the HPLC conditions used (50:50 acetonitrile/water as the mobile phase with a 1 ml/min flow rate), phenacyl derivatives of acetate and NAA had retention times of ~8.8 and ~18.0 min, respectively. The peak at ~13 min corresponds to the reaction byproduct p-bromophenacylchloride (Patience and Thomas, 1982), and the peak at ~15.5 min corresponds to the unreacted excess reagent p-bromophenacylbromide. The byproduct of the reaction, p-bromophenacylchloride, is nonreactive for esterification purposes and does not compete with the reagent. All other peaks appearing in the chromatogram are unknown but did not interfere with the detection of acetate and NAA. This method is highly sensitive, with concentrations of 10 ng of both acetate and NAA being within detection limits under the described conditions.

The recovery of acetate and NAA was 95 ± 2% and 93 ± 3%, respectively, without tissue homogenate and 89 ± 4% and 77 ± 3%, respectively, with brain tissue homogenate. The recovery rates were also determined using the internal standard method with nonradiolabeled standards of acetate and NAA, and similar results were obtained.

Figure 2 shows the acetate levels in brain and liver and the NAA levels in the brains of 20- to 21-day-old mice that received intragastric administration of varying doses of GTA. The animals were sacrificed 1 h after GTA administration. The increase of free acetate in the brain at a dose of 2.9 g/kg body weight of GTA was approximately 6-fold. This further increased to about 17-fold on a doubling of the dose but did not increase additionally at the higher dose of 7.2 g/kg body weight. The liver acetate levels showed a similar pattern of increase under these conditions. The NAA levels detected in the brain were not significantly changed even at the highest concentration of GTA administered, perhaps reflecting the relatively lower $K_m$ of acetyl CoA for aspartate N-acetytransferase, the biosynthetic enzyme of NAA (Madhavarao et al., 2003). The $ED_{50}$ of GTA, or the dose required to achieve half of the maximum level of acetate in the brain (8.19 µmol/g tissue), was calculated to be 4.389 g/kg animal weight, whereas in the liver, the value was 4.458 g/kg in 20- to 21-day-old mice.

Figure 3 shows the time courses for increases in acetate levels in the brains and livers and NAA levels in the brains of 20- to 21-day-old mice in response to a single dose (5.8 g/kg) of GTA. Free acetate levels in both liver and brain were increased about 16-fold at 1 h. The elevated level of acetate was maintained for up to 2 h, and the acetate levels at 1 and 2 h after GTA administration were found to be significantly higher than the control values ($P < 0.0001$), whereas at other time points, the differences were not significant compared with the control values based on Dunnett’s post hoc analysis. Eight hours following GTA administration, the brain acetate level remained elevated at about 2-fold, whereas the liver level was approaching normal. Brain acetate levels remained slightly but significantly higher at 12 h as compared with controls. The level of NAA in the brain at different time

![Fig. 1. HPLC elution patterns of phenacylster derivatives of brain extract of mouse fed 5.8 g/kg body weight GTA and sacrificed after 1 h (A), untreated control mouse (B), and standards, sodium acetate, and NAA (C). Acetate and NAA had retention times of ~8.8 and ~18.0 min, respectively.](image)

![Fig. 2. Brain and liver acetate levels and brain NAA levels 1 h after feeding varying doses of GTA. Mice 20 to 21 days old (~8 g body weight) were used in these studies. Values are mean ± S.D. of samples from three to five animals per group. *, P < 0.0001.](image)
intervals following GTA administration remained unchanged through the 12-h time course following GTA administration. The half-life of GTA-derived acetate was calculated for brain and liver tissue based on the level of acetate at 1 h (the time point at which maximum acetate level was attained) and at 4 h (the time point at which the level of acetate decreased to a comparable level with control). The values were found to be 70.8 min in the brain and 64.8 min in the liver.

Figure 4 compares the effectiveness of GTA with calcium acetate as a method for delivering additional acetate to the brain. Equimolar concentrations of GTA and calcium acetate were administered intragastrically to 20- to 21-day-old mice, and the animals were sacrificed after 1 h. The results show that GTA is significantly more effective than calcium acetate in increasing acetate levels in both brain and liver. This may reflect the ability of GTA to penetrate cell membranes readily due to its highly hydrophobic nature. It should be noted that GTA has 33.3% more acetate content than calcium acetate on an equimolar basis. The calcium acetate dose used in our study (26.5 mmol/kg) is equivalent to 3.86 g/kg GTA in terms of acetate content. From the dose-response study (Fig. 2), it is clear that 3.86 g/kg GTA delivers significantly higher levels of acetate to the brain than 26.5 mmol/kg calcium acetate.

Table 1 shows the concentrations of acetate and NAA in the brain and the level of acetate in the liver of mice at varying stages of development. The concentrations were computed by the internal standard method from peak area ratios using curves of acetate and NAA standards combined with tissue samples, as compared with tissue samples alone. The amount of free acetate in whole-brain extracts of adult mice used in these experiments was found to be 0.7 ± 0.05 μmol/g tissue, which is consistent with previous reports (Colon et al., 1987; Kiselevski et al., 2003). The NAA concentrations in brains of adult mice were found to be 8.8 ± 0.5 μmol/g tissue, which is also consistent with an earlier report (Tallan et al., 1956). As described earlier, NAA showed a substantial concentration increase during development (Tallan, 1957; Koller and Coyle, 1984; Florian et al., 1996), whereas we found that acetate is only moderately increased in the brains of adult mice as compared with developing mice.

The effect of GTA administration on the levels of acetate in brain and liver during different developmental stages of mice is given in Fig. 5. The results obtained show that the dose of GTA required to deliver a significant amount of acetate to the brain increases with age. For example, a dose of 1.45 g/kg animal weight is sufficient to cause a 5-fold increase in 7- to 8-day-old mice but only about a 2-fold increase in 13- to 14-day-old mice. No significant increase in brain acetate is detected under this condition in 17- to 20-day-old or adult mice. At a dose of 2.9 g/kg GTA, brain acetate levels increased approximately 17-fold in 7- to 8-day-old mice, 12-fold in 13- to 14-day-old mice, 7-fold in 17- to 20-day-old mice, and 5-fold in adult mice. The pattern of increasing acetate in the liver was similar to that in the brain.

To determine whether chronic GTA supplementation has overt toxic effects, a group of five 7-day-old mice were fed GTA orally two times a day until 25 days of age (2.9 g/kg animal weight until day 15 and after that 5.8 g/kg). At the end of the 25-day period, the mice were examined for apparent pathologies and were weighed. The GTA-fed animals survived the experimental period in good health. They had normal appearance and behavior throughout with an absence of diarrhea. At termination, their plasma was clear, and the animals did not develop fatty livers. Autopsies revealed normal-appearing stomach and intestine, and the data in Fig. 6 demonstrate that the GTA-fed animals exhibited a small increase in weight, possibly due to the increased caloric intake associated with GTA feeding (Bailey et al., 1989, 1991, 1993).

| Table 1 |
| Levels of acetate in brain and liver and of NAA in brain at different stages of development in mice (three to five animals per group) |
| Values given are means ± S.D. |
| Mouse age (days) | Acetate (μmol/g tissue) | NAA (μmol/g tissue) |
| Brain | Liver | Brain | Liver |
| 7/8 | 0.46 ± 0.03 | 0.5 ± 0.05 | 2.7 ± 0.5 |
| 13/14 | 0.44 ± 0.03 | 0.55 ± 0.05 | 4.5 ± 0.5 |
| 20/21 | 0.45 ± 0.05 | 0.52 ± 0.05 | 7.7 ± 0.5 |
| Adult | 0.7 ± 0.05 | 0.61 ± 0.05 | 8.8 ± 0.5 |
Discussion

The results presented here demonstrate two important issues. GTA is a more effective source for acetate delivery to the brain than calcium acetate. NAA levels are not increased when acetate levels are increased as high as 17-fold in the brain. Given the low toxicity of GTA and its Food and Drug Administration approval for human use, GTA represents a good candidate for use in the proposed acetate supplementation therapy for CD. Furthermore, GTA did not elicit any noticeable toxic effects and did not cause the overt gastrointestinal irritation associated with high doses of calcium acetate.

Earlier studies from our laboratory showed that there was nearly an 80% reduction in free acetate levels in the brains of ASPA−/− mice as compared with wild-type mice, but the levels in kidney and liver were not reduced relative to controls (Madhavarao et al., 2005). These findings demonstrate that some lipogenic pathways in the brain are unique and that in contrast to tissues such as liver, NAA is a significant source of free acetate in the brain during development. Furthermore, they demonstrate that myelin lipid synthesis in the brain derives a substantial portion of the requisite acetate from NAA via ASPA-mediated catalysis. Gene therapy using adeno-associated virus has been used in the tremor rat, a genetic model of CD, and ASPA activity was found to be expressed in CNS neurons following treatment. NAA levels were also reduced in the brain; however, motor functions in these animals remained unimproved (Klugmann et al., 2005). A major drawback of gene therapy is that ASPA protein is not expressed in oligodendrocytes, and myelin lipid synthesis remains dysfunctional. As such, we propose acetate supplementation with GTA as a therapy for Canavan disease, and the bases for the proposed use are summarized below.

First, myelination in the human CNS does not commence until early postnatal life, so postnatal diagnosis could be followed by immediate dietary acetate supplementation. Second, earlier studies have shown that free acetate is almost as effective as the acetyl moiety of NAA for fatty acid/lipid synthesis during myelination (D’adamo and Yatsu, 1966; D’adamo et al., 1968; Burri et al., 1991; Mehta and Namboodiri, 1995; Chakraborty et al., 2001). Third, in the developing rat, approximately 10% of radiolabeled acetate administered is incorporated into brain lipids, whereas about 5% is incorporated into lipids in the liver (Dhopeshwarker and Mead, 1973). Because oligodendrocytes are the cells that express aspartoacylase (Kirmani et al., 2002; Madhavarao et al., 2004), they are the target cells for acetate supplementation therapy in CD. The question of whether acetate levels are significantly increased in oligodendrocytes after GTA administration remains to be answered. However, the high $K_m$ values for acetate uptake systems in the blood-brain barrier (Terasaki et al., 1991) and in glial cells (Waniewski and Martin, 1998) suggest that a significant proportion of blood acetate would be delivered to the brain, where it could be taken up by glia, including oligodendrocytes. As noted above, in the developing rat, it has been shown that approximately 10% of the radiolabeled acetate administered systemically is incorporated into myelin lipids in the brain, demonstrating that a portion of the administered acetate is reaching oligodendrocytes where myelination takes place (Dhopeshwarker and Mead, 1973).

The superior effectiveness of GTA over calcium acetate for increasing brain acetate levels is of interest. GTA is thought to be at least partly hydrolyzed in the gut by the action of nonspecific esterases (Bach and Metais, 1970). However, our finding that brain acetate levels are significantly higher with GTA as opposed to calcium acetate on an equimolar basis cannot be accounted for by the increased (33.3%) acetate content of GTA. This suggests that a portion of GTA is transported unhydrolyzed from the gut to blood, from where
it can easily enter cells due to its hydrophobic nature. GTA was more effective at the earlier stages of development, possibly reflecting the lower activity levels of nonspecific esterases in the gut, due to their slower developmental profiles.

Regarding toxicity concerns of GTA, the Cosmetic Ingredient Expert Panel (Fiume, 2003) concluded that although there are no available reproductive and developmental toxicity data, GTA is metabolized to glycerol and acetic acid, which are not developmental toxins; hence, the use of GTA does not present a significant risk. GTA is also used as a food and drug additive and has been studied as a parenteral nutrient (Bailey et al., 1989, 1991, 1993). One potential problem with dietary acetate supplementation for CD infants is that GTA has a strongly bitter taste, and relatively large amounts would have to be administered orally on a twice daily basis. It is possible that sweeteners could be used to overcome this difficulty.

No significant increases in NAA concentrations were observed in the present investigation, even when brain acetate levels were increased 17-fold. This finding may have clinical significance for CD patients, who are reported to have 10- to 100-fold higher excretion of NAA in the urine than normal (Kvittingen et al., 1986; Hagenfeldt et al., 1987; Matalon et al., 1988) and a 0- to 3-fold increase in NAA levels in the brain as compared with normal individuals (Wittsack et al., 1996). One hypothesis on the pathogenesis of CD is that the increased brain NAA concentrations result from a lack of catabolic activity and the excess NAA plays an etiological role in the disease, possibly due to brain edema (Baslow, 2003). If this hypothesis is found to be correct, an increase in brain NAA concentrations during acetate supplementation therapy would exacerbate the pathology. Given our present findings that NAA levels remain unchanged with GTA feeding, increased brain edema should not be a major concern of acetate supplementation therapy for CD. A possible explanation for the lack of increase in NAA concentrations is that the biosynthetic enzyme for NAA has a relatively low Km for acetyl-CoA (Madhavarao et al., 2003). This would mean that the NAA synthetic enzyme would be saturated at relatively low concentrations of acetyl-CoA, thus limiting NAA production when acetate concentrations were increased. Furthermore, product feedback inhibition by the increased levels of NAA in CD patients should help maintain NAA synthesis at lower levels (Moreno et al., 2001). In addition, it is noteworthy that our simple HPLC analytical technique can detect NAA at nanogram levels, providing a simple and inexpensive method which can be used to clinically diagnose CD.

Finally, additional optimization studies are required before the initiation of preclinical trials in CD mice. For example, the optimal dose and dosing frequency required to maintain increased acetate levels in the brain need to be determined. The minimally increased level of acetate in the brain that is required to correct the acetate deficit in oligodendrocytes remains unclear. One immediate consequence of continuous GTA treatment involves possible acidosis caused by continuous CO2 production via the oxidation of acetate. This issue should also be addressed before large-scale clinical trials can begin. However, it is encouraging to note that the acetate supplementation for CD could be reduced or discontinued by the end of the second year after birth because myelin synthesis is nearly complete at that stage of development.

References


synthesis rate in the human brain by $^{13}$C MRS and [1-$^{13}$C] glucose infusion

Use of GTA for Acetate Supplementation to the Brain


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