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N-butyldeoxynojirimycin delays motor deficits, cerebellar microgliosis, and Purkinje cell loss in a mouse model of mucolipidosis type IV

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ABSTRACT

Mucolipidosis type IV (MLIV) is a lysosomal storage disease exhibiting progressive intellectual disability, motor impairment, and premature death. There is currently no cure or corrective treatment. The disease results from mutations in the gene encoding mucolipin-1, a transient receptor potential channel believed to play a key role in lysosomal calcium egress. Loss of mucolipin-1 and subsequent defects lead to a host of cellular aberrations, including accumulation of glycosphingolipids (GSLs) in neurons and other cell types, microgliosis and, as reported here, cerebellar Purkinje cell loss. Several studies have demonstrated that *N*-butyldeoxynojirimycin (NB-DNJ, also known as miglustat), an inhibitor of the enzyme glucosylceramide synthase (GCS), successfully delays the onset of motor deficits, improves longevity, and rescues some of the cerebellar abnormalities (e.g., Purkinje cell death) seen in another lysosomal disease known as Niemann-Pick type C (NPC). Given the similarities in pathology between MLIV and NPC, we examined whether miglustat would be efficacious in ameliorating disease progression in MLIV. Using a full mucolipin-1 knockout mouse (*Mcoln1*$^{-/-}$), we found that early miglustat treatment delays the onset and progression of motor deficits, delays cerebellar Purkinje cell loss, and reduces cerebellar microgliosis characteristic of MLIV disease. Quantitative mass spectrometry analyses provided new data on the GSL profiles of murine MLIV brain tissue and showed that miglustat partially restored the wild type profile of white matter enriched lipids. Collectively, our findings indicate that early miglustat treatment delays the progression of clinically relevant pathology in an MLIV mouse model, and therefore supports consideration of miglustat as a therapeutic agent for MLIV disease in humans.

1. Introduction

Mucolipidosis type IV (MLIV, OMIM #252650) is an autosomal recessive, lysosomal storage disorder (Bach et al., 1975; Bargal et al., 2000; Tellez-Nagel et al., 1976). Typically, the disease clinically presents at 1–2 years of age and over time leads to severe and progressive intellectual disability, motor and speech deficits, progressive retinopathy leading to blindness, systemic pathologies, and a shortened lifespan (Amir et al., 1987; Schiffmann et al., 2014; Venugopal et al., 2007). There is currently no corrective therapy or cure for MLIV. The prevalence of MLIV is relatively high in the Ashkenazi Jewish population with an estimated carrier frequency of 1:100, but can also occur in the general population (Altarescu et al., 2002; Bargal et al., 2001, 2000; Edelmann et al., 2002). The disease results from mutations in the gene encoding mucolipin-1 (*MCOLN1*), a 65 kDa protein also known as TRPML1 that serves as a transmembrane transient receptor potential (TRP) channel found in the limiting membrane of late endosomes and lysosomes (Bach, 2005; Bargal et al., 2000; Ye et al., 2004). *MCOLN1* is localized to chromosome 19p13.2–13.3 with both major and minor MLIV-causing mutations identified in *MCOLN1*, as well as over 20 independent mutations (Bargal et al., 2001, 2000; Slaugenhaupt et al., 1999; Sun et al., 2000; Venugopal et al., 2007; Wakabayashi et al., 2011). These mutations range from causing reduced mRNA expression to yielding nonfunctional protein (Sun et al., 2000; Wakabayashi et al., 2011). Mucolipin-1 is ubiquitously expressed with highest expression in the brain and kidney (Bach, 2005; Cheng et al., 2010) and its loss leads to systemic disease accompanied by severe neurological pathology.

The function of mucolipin-1 is not fully understood, but it is known that it serves a critical role in pH regulation and as a nonselective ion channel permeable to calcium, iron, and other cations (Bach, 2005;

Abbreviations: NB-DNJ, N-butyldeoxynojirimycin; GCS, glucosylceramide synthase; GSL, glycosphingolipid; CMH, ceramide monohexoside; CDH, ceramide dihexoside; GBA2, glucocerebrosidase; NPC, Niemann-Pick type C; SRT, substrate reduction therapy.

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Cheng et al., 2010; Dong et al., 2009; Dong et al., 2008; Soyombo et al., 2006). Endosomal/lysosomal calcium egress through this TRP channel appears to have an important function in key cellular processes requiring a local, lysosomal calcium source, such as the fusion of organelles during autophagy and lysosomal exocytosis (Curcio-Morelli et al., 2010; LaPlante et al., 2006; Lima et al., 2012; Medina et al., 2015). The loss of mucolipin-1 and resulting defects in these and possibly other processes lead to a host of MLIV pathologies, including storage of cellular substrates like glycosphingolipids (GSLs) normally targeted to the lysosome for degradation, dysregulation of lysosomal pH, and the build-up of autophagosomes (Bach et al., 1975; Micsenyi et al., 2009; Venkatachalam et al., 2008; Venugopal et al., 2007; Wong et al., 2015; Ye et al., 2004). In addition, loss of mucolipin-1 secondarily leads to other abnormalities such as widespread gliosis, dysmyelination, and Purkinje cell abnormalities (Grishchuk et al., 2014; Micsenyi et al., 2009; Schiffmann et al., 2014) and death as further reported here. Numerous MLIV disease models have been generated that recapitulate cellular pathologies, including murine, *Drosophila*, and *C. elegans* models (Lima et al., 2012; Schaheen et al., 2006; Venkatachalam et al., 2008; Venugopal et al., 2007). The MLIV mouse model (*Mcoln1*$^{-/-}$ mice) displays progressive behavioral and locomotor deficits, including hindlimb clasping, abnormal gait, and cognitive impairment (Grishchuk et al., 2014; Venugopal et al., 2007). As the mechanism linking loss of mucolipin-1 to these abnormalities is not fully established, this knowledge gap yields a major obstacle to developing a direct therapy for this disorder.

Substrate reduction therapy (SRT) has shown success in certain lysosomal diseases, such as Type 1 Gaucher, where enzyme replacement therapy (ERT) is not appropriate for all patients, and Sandhoff and Niemann-Pick type C (NPC) diseases where ERT or gene replacement therapy is not currently available (Aerts et al., 2006; Cox, 2005; Di Rocco et al., 2012; Pastores, 2006; Pastores and Barnett, 2003; Patterson et al., 2007; Stein et al., 2012; Tallaksen and Berg, 2009; Wortmann et al., 2009; Zervas et al., 2001b). SRT aims to improve overall cellular function by reducing the synthesis of those substrates which accumulate as storage (Aerts et al., 2006; Cox, 2005). These studies have shown success particularly with one compound, *N*-butyldeoxynojirimycin (NB-DNJ), otherwise known as miglustat, a small imino sugar that is an inhibitor of the GSL synthetic enzyme known as glucosylceramide synthase (GCS) (Elstein et al., 2004; Jeyakumar et al., 1999; Pastores, 2006; Pastores and Barnett, 2003; Platt et al., 1997a; Platt et al., 1994; Stein et al., 2012; Zervas et al., 2001b). Located in the ER and Golgi, GCS catalyzes the first committed step along the GSL pathway (Platt et al., 1997b). In the context of SRT, this enzyme acts upstream of substrates accumulating in type 1 Gaucher and Sandhoff diseases and contributing to storage in NPC disease (Pastores and Barnett, 2003; Platt et al., 1994; Zervas et al., 2001a, 2001b). Importantly, in murine and feline NPC models, miglustat successfully delayed Purkinje cell loss, cerebellar gliosis, the progression of motor deficits linked to cerebellar pathology, and improved longevity (Davidson et al., 2009; Stein et al., 2012; Zervas et al., 2001b). Given these findings, we hypothesized that miglustat may reduce the cerebellar abnormalities in MLIV and could represent a viable therapy that may improve the quality of life in MLIV patients. In order to investigate this hypothesis, we examined the effects of miglustat in *Mcoln1*$^{-/-}$ mice (Venugopal et al., 2007). We found that miglustat successfully delays motor deficits, improves the profile of specific lipid classes in the cerebellum, and delays cerebellar microgliosis and cerebellar Purkinje cell loss. We believe these data indicate that miglustat warrants further investigation as a treatment for MLIV patients.

2. Materials and methods

2.1. Mouse generation and administration of miglustat

Heterozygous (*Mcoln1*$^{+/-}$) mice were crossed to obtain littermate wild type (*Mcoln1*$^{+/+}$) and mutant (*Mcoln1*$^{-/-}$) mice, which were then genotyped prior to postnatal day 10 (P10) (Venugopal et al., 2007). Cohorts of male and female *Mcoln1*$^{+/+}$ and *Mcoln1*$^{-/-}$ littermate mice were then randomly assigned for treatment with miglustat or saline vehicle starting at P10. Using a miglustat solution of 30 mg/ml saline, mice received 300 mg miglustat/kg body weight or the equivalent volume of normal saline. These were administered daily via intraperitoneal (IP) injection from P10-P21, followed by IP injection three times a week after P21 and until the end of the study. A total of 47 mice were used in this treatment study: WT saline n = 14, WT miglustat n = 13, *Mcoln1*$^{-/-}$ saline *n* = 8, *Mcoln1*$^{-/-}$ miglustat treated n = 12. Additional mice were used as untreated *Mcoln1*$^{-/-}$ controls for western blot analyses and CD68 counts following the conclusion of the drug treatment cohort. All studies were performed in consideration of high standards of scientific rigor (Landis et al., 2012) and in accordance with the Albert Einstein College of Medicine's IACUC and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral studies

Behavioral assessments were made at timepoints selected to represent pre-symptomatic (7–15 for open field/object placement, 15–16 weeks for balance beam), early symptomatic (26 weeks), and end-stage disease (32 weeks) based on previous reports in *Mcoln1*$^{-/-}$ mice (Grishchuk et al., 2014; Micsenyi et al., 2009; Venugopal et al., 2007). Mice were given a randomized number to blind the tester to genotype and treatment, and their tails color-coded per cage during testing to represent each blinded number. All behavioral work was performed within the Albert Einstein College of Medicine Animal Phenotyping Core Facility and according to detailed published protocols (Dere et al., 2007; Ennaceur and Delacour, 1988; Stanley et al., 2005).

2.2.1. Open field

Mice were placed in a 41 × 41 cm open field testing box devoid of objects with orientation markers pasted to the sides of the box. Biobserve video tracking software was used to track and quantify the mouse's movements within the arena, including the total track-length explored, entries into the center arena, time spent in the center arena, and track-length in the center arena. The center of the arena was set at 17 × 17 cm for each trial. The number of rears was manually counted in a blinded fashion to the tester, with a “rear” being considered when the mouse lifted its body upward and both front paws were elevated from the floor. The task was performed over a 6 min window and analyzed in both 3 min bins and the total 6 min.

2.2.2. Object placement

Mice were placed in the open field box containing 2 identical objects. The amount of time spent exploring each object was manually timed over a 5 min window. The mouse was then removed from the arena for a 16 min window, during which one object was moved to a new location. The mouse was then placed back in the arena and the amount of time spent at each object timed manually over a 3 min testing window, with exploration of the objects including sniffing and/or touching the object in question. A preference for the newly placed object was deemed as greater than or equal to 55% exploration of the novel object compared to the unmoved object.

2.2.3. Balance beam

Three beams were utilized, 30 mm (wide), 24 mm (medium), and 18 mm (narrow) diameter beam. Beams were raised using tripods and stabilized, with hides available at the end of the beam to promote passage across the beam. A light gradient was generated to stimulate the mouse moving from the start (bright) of the beam to the end of the beam (dark). Covered bins were located underneath the beams as a safety precaution. Prior to first testing, mice were trained on a 3 in. wide-set beam to promote passage across the beam. During testing,