Glycomics & Lipidomics

Extended Abstract

A new role for glycolipids in Niemann-Pick C

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Abstract

Niemann-Pick type C disease (NPCD) is a devastating neurodegenerative condition most commonly due to mutations in endolysosomal NPC1. Mutations in NPC1 are associated with impaired endocytic transport via decreased endolysosomal calcium release. Endocytosis and luminal calcium are dependent on correct endolysosomal acidification and have been found to be controlled by glycolipids in neurons, melanocytes, Gaucher disease, plant vacuoles and C. elegans. NB-DNJ (miglustat) has approval for the treatment of NPCD as a GlcCer synthase inhibitor to lower lysosomal GlcCer. However, increased brain GlcCer when administered to animal models has led to wide speculation of off-target inhibition of non-lysosomal GlcCer (GBA2) breakdown. Off target inhibition of GBA2 was recently strengthened by the utility of a more specific GBA2 inhibitor AMP-DNM as well as GBA2 knockout in NPCD mice. Several studies have shown increased pH in NPC cell culture models. We show here that disrupted endocytic trafficking in NPCD cell culture models is associated with increased endolysosomal pH using lysosensor yellow blue to label all acidic compartments. To study these phenomena we examined the effects of U18666A on endolysosomal pH and glycolipid transport. NPCD cell culture models were found to have increased endolysosomal pH and inhibition of nonvesicular glucosylceramide (GlcCer) but not GalCer transport. In contrast, inhibiting non-lysosomal glucocerebrosidase (GBA2) decreased endolysosomal pH in normal cells, reversed increased endolysosomal pH and restored disrupted BODIPY-LacCer trafficking in NPCD fibroblasts.

Niemann-Pick type C (NPC) disease is a cholesterol lipidosis caused by mutations in NPC1 and NPC2 gene loci. Most human cases are caused by defects in NPC1, as are the spontaneously occurring NPC diseases in mice and cats. NPC1 protein possesses a sterol-sensing domain and has been localized to vesicles that are believed to facilitate the recycling of unesterified cholesterol from late endosomes/lysosomes to the ER and Golgi. In addition to accumulating cholesterol, NPC1-deficient cells also accumulate gangliosides and other glycosphingolipids (GSLs), and neuropathological abnormalities in NPC disease closely resemble those seen in primary gangliosidoses. These findings led us to hypothesize that NPC1 may also function in GSL homeostasis. To evaluate this possibility, we treated murine and feline NPC models with N-butyldeoxynojirimycin (NB-DNJ), an inhibitor of glucosylceramide synthase, a pivotal enzyme in the early GSL synthetic pathway. Treated animals showed delayed onset of neurological dysfunction, increased average life span (in mice), and reduced ganglioside accumulation and accompanying neuropathological changes. These results are consistent with our hypothesis and with GSLs being centrally involved in the pathogenesis of NPC disease, and they suggest that drugs inhibiting GSL synthesis could have a similar ameliorating effect on the human disorder.

Niemann-Pick type C (NPC) disease is a fatal neurodegenerative disorder caused most commonly by a defect in the NPC1 protein and characterized by widespread intracellular accumulation of unesterified cholesterol and glycosphingolipids (GSLs). While current treatment therapies are limited, a few drugs tested in Npc1(-/-) mice have shown partial benefit. During a combination treatment trial using two such compounds, N-butyldeoxynojirimycin (NB-DNJ) and allopregnanolone, we noted increased lifespan for Npc1(-/-) mice receiving only 2-hydroxypropyl-beta-cyclodextrin (CD), the vehicle for allopregnanolone. This finding suggested that administration of CD alone, but with greater frequency, might provide additional benefit.

Administration of CD to Npc1(-/-) mice beginning at either P7 or P21 and continuing every other day delayed clinical onset, reduced intraneuronal cholesterol and GSL storage as well as free sphingosine accumulation, reduced markers of neurodegeneration, and led to longer survival than any previous treatment regime. We reasoned that other lysosomal diseases characterized by cholesterol and GSL accumulation, including NPC disease due to NPC2 deficiency, GM1 gangliosidosis and mucopolysaccharidosis (MPS) type IIIA, might likewise benefit from CD treatment. Treated Npc2(-/-) mice showed benefits similar to NPC1 disease, however, mice with GM1 gangliosidosis or MPS IIIA failed to show reduction in storage.

Treatment with CD delayed clinical disease onset, reduced intraneuronal storage and secondary markers of neurodegeneration, and significantly increased lifespan of both Npc1(-/-) and Npc2(-/-) mice. In contrast, CD failed to ameliorate cholesterol or glycosphingolipid storage in GM1 gangliosidosis and MPS IIIA disease. Understanding the mechanism(s) by which CD leads to reduced neuronal storage may provide important new opportunities for treatment of NPC and related neurodegenerative diseases characterized by cholesterol dyshomeostasis.

Starting at postnatal day 7 (P7) and thereafter, NPC1 knock-

out mice (NPC1(-/-)) and wild type controls (NPC1(+/+)) were injected with cyclodextrin/allopregnanolone weekly. Additionally, a daily miglustat injection started at P10 until P23. Starting at P23 the mice were fed powdered chow with daily addition of miglustat. The sham group was injected with 0.9% NaCl at P7, thereafter daily starting at P10 until P23, and fed powdered chow starting at P23. For corneal examination, in vivo confocal laser-scanning microscopy (CLSM) was performed one day before experiment was terminated.

Excised corneas were harvested for lipid analysis (HPLC/MS) and electron microscopy. In vivo CLSM demonstrated a regression of hyperreflective inclusions in all treated NPC1(-/-)mice. The findings varied between individual mice, demonstrating a regression, ranging from complete absence to pronounced depositions. The reflectivity of inclusions, however, was significantly lower when compared to untreated and sham-injected NPC1(-/-) mice. These confocal findings were confirmed by lipid analysis and electron microscopy. Another important CLSM finding revealed a distinct increase of mature dendritic cell number in corneas of all treated mice (NPC1(-/-) and NPC1(+/+)), including sham-treated ones

This work is partly presented at 4th Glycobiology World Congress September 17-19, 2018

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