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Niemann–Pick type C pathogenesis and treatment: from statins to sugars

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Abstract

The isolation of the causative genes for Niemann–Pick type C disease, a panethnic lysosomal lipid storage disorder, has provided models of how sterols and other lipids such as glycosphingolipids traverse the membranes of eukaryotic cells. Unfortunately, these molecular advances have yet to reciprocate with a cure for this devastating neurodegenerative disorder where neuronal replenishment will most likely yield the greatest benefit. In the meantime, stabilizing treatment strategies based on the removal of presumably toxic metabolites are in place. For example, the small molecule inhibition of glucosylceramide synthase by miglustat limits ganglioside accumulation and is now the only approved treatment of Niemann–Pick type C. In addition, 2-hydroxypropyl- β -cyclodextrin, a lipid chelator, relieves the lysosomal to endoplasmic reticulum blockage and markedly increases the life expectancy of the murine model. Ultimately, these strategies, targeting the primary biochemical lesion in these cells, and others will likely be combined to provide a synergistic cocktail approach to treating this disease.

Keywords

cholesterol; neurodegeneration; Niemann–Pick type C; sphingolipid

Neurodegenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis, multiple sclerosis and Parkinson's disease represent some of the leading causes of disability in modern populations and a significant burden to western society (e.g., 7% of the total attributable cost of all illness in Canada). A total of 5 million Americans have AD, a number that has doubled since 1980 and is expected to be as high as 13.4 million by 2050 [101]. Very few neurodegenerative conditions are curable and most worsen over time. Even rare monogenic conditions, where the biochemical and genetic etiology is well defined, are generally fatal. However, Mendelian disorders, especially when of high penetrance, can be very informative with regard to disease mechanism and can even dictate therapeutic strategies for related polygenic pathologies (e.g., defects in the LDL receptor and hypercholesterolemia). Niemann–Pick type C (NP-C) disease, is a panethnic lysosomal lipidosis that results in cerebellar impairment and death, presumably as a consequence of defective lipid transport [1,2]. As the disease progresses, tissue lipids accumulate and neurological, gastrointestinal, liver and respiratory problems ensue (Table 1). NP-C disease is a tauopathy similar to AD and thus we speculate that protocols that arrest NP-C may also limit the course of related, but etiologically undefined, neurodegenerative syndromes.

Historical perspective of NP-C disease: the search for defective genes

In 1984, Peter Pentchev and colleagues made the telling discovery that the biochemical hallmark of NP-C disease is lysosomal accumulation of LDL-derived, unesterified cholesterol, and consequently misregulated sterol biosynthesis, uptake and esterification [3]. Genetic studies (mainly cell complementation approaches) identified two principle loci for the disease – the most prevalent of which was localized to human chromosome 18. A heroic collaboration between afflicted families, family-run foundations (notably the Ara Parseghian Medical Research Foundation, AZ, USA) and researchers at NIH, led by Pentchev, identified defects in the human [4] and murine [5] *NPC1* genes as being responsible for the most common form of NP-C disease. The second (but much rarer) causative locus for this disease, *NPC2*, was later isolated in a proteomics survey of the lysosome [6].

The *NPC1* and *NPC2* genes defective in NP-C disease encode an approximately 13-pass transmembrane domain protein and a soluble low-molecular-weight luminal protein, respectively [7]. We have hypothesized that the NPC1 protein is a cellular rheostat that transports lipids and interacts with cellular signaling pathways to optimize membrane fluidity [1]. Both NP-C proteins have been proposed as cholesterol pumps or chaperones, in part because they both possess lipid-binding domains and have even been cocrystallized with sterol [8,9]. However, the affinity for cholesterol of NPC1 is orders of magnitude lower than of NPC2 [10,11]. NP-C disease is further typified by the accumulation of sphingolipids [12], and thus cholesterol binding to NPC1 may act catalytically, as opposed to bulk transfer or solubilization.

Niemann–Pick type C disease may be unusual with regard to the abundance of model systems that are available to study its underlying mechanisms. The NP-C genes are conserved throughout eukaryotic evolution to the extent that expression of the orthologous genes, *NCR1* and *NPC2* from budding yeast (*Saccharomyces cerevisiae*) complements NPC1 and NPC2 lesions in mammalian cells, respectively [13,14]. The genes for this disorder are undergoing study in organisms as diverse as nematodes, fruit flies, sea squid, cats and mice [15]. The *Npc1*^{-/-} BALB/c strain of mice (defined by an early truncation of the NPC1 protein and thus a null allele [5]) have all the hallmarks of severe human disease; including detectable Purkinje cell death at 5 weeks of age, onset of neurological symptoms at 7 weeks and death at approximately 10 weeks. Human NP-C disease this aggressive is the extreme spectrum of this invariably fatal disorder, raising the possibility that these mice are not the ideal model for therapeutic strategies. Alternate murine models are under development (e.g., the *Npc1*^{nmf164} strain from Jackson Laboratories was an outcome of a chemical mutagenesis screen and exhibits later disease onset and slower progression), as is an excellent feline model that may more closely exhibit the human syndrome disease progression and may thus be useful for treatment development or affirmation [16,17].

How & why do some cells lacking the Niemann–Pick type C genes die?

The major and fatal insult of NP-C disease is neurologic; symptoms include vertical gaze palsy, ataxia, dystonia, dysphagia, seizures, cataplexy, dementia and psychiatric illness [18]. The age of onset of these neurological conditions has a major impact on the severity of the disease; however, the progression remains essentially the same [19]. The question of what is happening biochemically at the neuronal level to cause these symptoms remains unclear. *NPC1* and *NPC2* are expressed from embryo to adulthood in most tissues, including the cerebellum [20]. Purkinje neurons, stacked on top of each other, creating the Purkinje layer in the cerebellum are responsible for movement and coordination. In NP-C disease, the Purkinje cells die in a characteristic pattern that exacerbates with age and disease state [21]. Effective treatments have demonstrated reduction in Purkinje cell loss indicative of their

importance in disease pathology [22]. Prior to the appearance of a neurologic phenotype, the NPC1 protein is found at the terminal fields of axons and dendrites in neurons demonstrating the earliest signs of neurodegeneration [23]. Although Purkinje cell loss has long been thought of as the major determinant of the neurological phenotype seen in NP-C disease, recent studies involving conditional knockdown of NPC1 in Purkinje cells have shown otherwise. Mice lacking NPC1 specifically in Purkinje cells exhibited Purkinje cell death in an age-specific manner, resulting in ataxia and motor problems, but with no impact on survival or weight loss, relative to treatment controls [24]. This strengthens the case for the involvement of other cells of the neural system, such as glia and astrocytes [23,25]. Thus, the exact neurologic insult of NP-C disease remains to be deciphered, but the fact remains that effective treatment of this disease must include replenishing or rescuing the neuronal loss, specifically the Purkinje cells [26].

Is there a toxic metabolite?

Niemann–Pick type C disease is a heterogeneous collection of metabolic phenotypes with a panoply of accumulations, mainly in the lysosomal/endosomal system (Table 1). The challenge is to establish a priority (if any exists) to these compounds with the assumption that one of them is causal to the disease. Strikingly, the majority of these metabolites are potent mediators of cell signaling and cell death, thus it seems reasonable that limiting their accumulation would be beneficial. Given the wide array of metabolites and indeed the relative obscurity of a known substrate for the NPC transporters, it is also plausible that the toxic metabolite remains to be identified. Metabolomic screens may ultimately clarify this, as well as provide surrogate markers of the disease – a currently unmet need in treating and monitoring this syndrome.

Rational approaches to treatment: seemed like a good idea at the time?

It was a forlorn hope that the identification of the causative defective gene for this devastating disorder would present an obvious and rapid therapeutic target. It was immediately clear, for example, that enzyme replacement, a strategy in place for treatment of several lysosomal disorders, was not possible with a membrane protein such as NPC1. Instead, strategies must target saving and replenishing the neurons of the cerebellum, reducing toxic metabolites and repairing the faulty transport mechanism (Figure 1). At present, the most effective strategies rely on limiting the damage.

Cholesterol-lowering drugs

Initially, the theoretical approach to NP-C disease treatment seemed simple; lower the excess cholesterol and thus reverse the disease. This simple ideology was strengthened by the fact that many, highly effective, cholesterol-lowering medications existed. The molecular and biochemical characterization of the NP-C proteins further supported this strategy; *NPC1* and *NPC2* encode proteins that bind cholesterol in a variety of assays. Cholesterol is also widely known to play a key role in proper neuronal functioning and the hope was that ameliorating the toxic effect of the molecule would be the answer in treatment [27]. In 1993, Patterson *et al.* found that cholesterol-lowering agents reduced hepatic and serum cholesterol levels in a small number of human NP-C patients with no impact on neurological symptoms [28]. Several years later, the approach was repeated in a NP-C mouse model, again with no detectable neurological benefit [29,30]. Although total body cholesterol levels can be very effectively reduced in this disease state, this does not affect the brain and thus the neurological symptoms of the disease persist. Surprisingly, genetic approaches to manipulating cholesterol homeostasis in the brain were also without consequence in terms of disease severity [31]. Moreover, very few changes in sterol metabolism were observed in yeast or nematode models of loss-of-function of the NP-C

orthologs, suggesting that the primordial, and perhaps basic, role of the NP-C protein was not transport of sterols [15]. Although this evidence suggests that cholesterol may not be the key offending metabolite in NP-C disease, it would be surprising if it is merely an innocent bystander.

Sphingolipid-lowering drugs

Later approaches focused on reducing other toxic metabolites (e.g., sphingomyelin, sphingosine, cerebroside and other complex glycosphingolipids) by substrate reduction [32]. Miglustat (*N*-butyldeoxynojirimycin, NB-DNJ, Zavesca,) an approved intervention for Gaucher's disease, inhibits glucosylceramide synthase, a key component of the glycosphingolipid biosynthetic pathway [33]. Both the feline and murine models responded positively to treatment, resulting in reduced ganglioside accumulation, reduced adverse neuronal pathology, delayed neuronal dysfunction and a significant (25%) increase in lifespan from 67 ± 1 (untreated) to 89 ± 5 days [34]. Several clinical trials have since been conducted in humans using miglustat demonstrating mild clinical improvement or stabilization, with greater impact on earlier diagnosed populations. Miglustat is the only currently approved therapy for NP-C disease and clearly slows disease progression with limited side effects [35–38]. Nevertheless, it is likely to be a necessary component of any NP-C treatment cocktail, if only because most patients are taking this drug already. The efficacy of miglustat highlights the importance of glycosphingolipids in NP-C disease pathogenesis, because similar treatments to reduce cholesterol did not show such impact.

End product replacement therapy

End product replacement therapy targets the final products of a metabolic pathway that are deficient in a disease state. Cholesterol is the obligate precursor in the biosynthesis of neurosteroids required for brain development, particularly through neuronal growth and survival [39]. Human fertility is apparently normal in patients; however, NP-C mice are infertile with underdeveloped reproductive organs and lower levels of the neurosteroid, allopregnanolone, in their brains [40,41]. Owing to these discoveries, NP-C mice were treated with allopregnanolone, with the hypothesis that increasing the levels of this hormone might rectify the deficiency as well as preserve myelin [42]. Drug administration increased Purkinje cell survival and surprisingly reduced ganglioside accumulation [41]. When the treatment was delivered in the drinking water, survival increased from 67 to 80 days, and when the treatment was administered via skin pellets, the lifespan was increased to 124 days. This experiment was also the first to initiate treatment at postnatal day 7 (equivalent to early gestation in humans), a time when drug penetration to the brain is greater than in the adult mouse. Strikingly, treatment administered once, at day 7, was enough to confer a marked benefit to the animals [41].

Sterol-binding drug therapy

Skepticism surrounds allopregnanolone treatment; it is the only strategy that targets end product replacement, while in fact the conferred benefit is predominantly owing to the vehicle's ability to target the intracellular lipid storage defect. A serendipitous outcome of the allopregnanolone trials in mice was the subsequent observation that treatment with the vector, 2-hydroxypropyl- β -cyclodextrin (CYCLO), was just as effective in increasing lifespan [31]. Indeed, later studies indicate the vector was primarily responsible for the observed therapeutic impact [43]. Cyclodextrins are composed of cyclical oligosaccharides with a hydrophilic exterior and hydrophobic interior, and are thus ideal chelators of sterols [44]. The lipid-binding ability of these molecules enables the sequestered lysosomal lipid to flow into the cytosolic pool in NP-C animals [31,45] and cells [46], relieving the cellular burden of lipids. A single dose, administered at day 7, is necessary and sufficient to increase

cellular cholesteryl esters, suppress cholesterol synthesis, decrease inflammatory markers and confer significant improvement in neurodegeneration and increased lifespan from 84 to 120 days [45]. Interestingly, CYCLO has no effect on the same process in wild-type animals [45]. To date, CYCLO treatment has shown the greatest improvements of any treatment in animals and is the first result to suggest that NP-C disease could be halted. The movement of cholesterol from lysosome to endoplasmic reticulum is the favored point of action [45,46], although its precise mechanisms are still to be determined.

NP-C replacement therapy

This group of therapies includes treatment avenues directed at replacing faulty NP-C pathways with new ones, through enzyme replacement, bone marrow transplantation, stem cell therapy or gene therapy. Bone marrow transplantation resulted in improved visceral organ function, but did not impact the neurologic disease [47]. When bone marrow-derived stem cells were injected in the cerebellum of NP-C mice, some markers of inflammation were controlled along with the reduced activation of astrocytes and microglia (cells that promote neuronal apoptosis) [48]. High levels of nitric oxide have been found in neural stem cells isolated from NP-C animals. These increased levels impair the self-renewal process of the cells and could play a key role in using stem cell therapy in NP-C disease [49]. Lifespan in mice increased from 73 to 90 days through neural stem cell implantation, although no effect on weight loss or neurological symptoms were seen [50]. Gene therapy seems to be a vital player in this devastating disease, but the challenging problems associated with effective delivery, long-term expression and avoidance of an immune response must still be overcome [26]. These techniques, particularly enzyme replacement, may be more effective in treatment of NP-C type 2 disease because the NPC2 protein is soluble. The loss of neuronal cells is a critical problem in the treatment of NP-C disease, and thus efforts to replenish this resource may be essential in concert with other therapies.

Cell-signaling targets

Signaling cascades are the infrastructure to all processes within the cell, consisting of many components that are altered in disease states. Although these cascades are often complex, if the pathway is well discerned, it allows for specific interventions (Figure 1). For example, T0901317, a potent and selective agonist of nuclear hormone receptors (LXR α and LXR β , PXR), whose native ligands are oxysterols, significantly increased cholesterol excretion from the brain and prolonged life [51]. Rab9 is a small GTPase protein involved in membrane trafficking, and has been shown to correct glycosphingolipid trafficking defects when upregulated [52]. This upregulation also corrects in NPC2 cell lines and NPC1 neurons [53]. It is thought that Rab9 may be sequestered or inactive in NP-C disease, causing the hallmark disease phenotype of lipid accumulation in the lysosomal compartment [54]. The currently proposed model suggests that lipid accumulation causes the inhibition of protein kinase C, which hyperphosphorylates the intermediate filament vimentin, inducing the aggregation and entrapment of Rab9 leading to endosomal dysfunction [55]. Overexpression of Rab9 alleviated ganglioside storage in the murine NP-C model resulting in a 22% increase in lifespan [56].

Another key pathway that plays a role in several phenotypes shared by NP-C and AD is the glycogen synthase kinase 3 β (GSK3 β) cascade. GSK3 β has been heavily implicated in the pathogenesis of AD, specifically through the increased activity of GSK3 β causing hyperphosphorylation of tau, which creates the neurofibrillary tangles seen in both AD and human NP-C. GSK3 β is a drugable target and significant reduction in tau phosphorylation has been attained with lithium or other GSK3 β inhibitors [57]. CDK5 is also involved in the tau hyperphosphorylation pathway. Overexpression of CDK5 causes cytoskeletal

abnormalities that lead to neurological disorders such as NP-C, possibly through the formation of neurofibrillary tangles in the human brain [58]. Accordingly, inhibition of CDK5 by drugs such as R-Roscovetine [59] ameliorated NP-C disease symptoms in a short-term study [60].

Alternate therapies

Inflammation has been shown to play a role in the pathogenesis of neurological disorders, including NP-C disease, suggesting a potential intervention through the use of NSAIDs and antioxidants. The NSAID, ibuprofen conferred improved longevity and reduced markers of inflammation [61]. Consistent with what is observed in other therapeutic regimes, a greater benefit was demonstrated in a combination treatment of ibuprofen and miglustat.

Nutritional treatments create an important adjunct to specific treatments discussed. Many therapies have been investigated in the NP-C disease model systems and are currently implemented by patients. Dietary cholesterol restriction was an early intervention but experiments conducted in the NP-C feline model indicated no effect on lifespan or symptoms [62]. Similarly, increasing dietary cholesterol did not impact brain levels or limit the lifespan of NP-C mice [63]. Much attention has been paid to antioxidants to reduce the subcellular stress in NP-C patients as NP-C fibroblasts have higher levels of reactive oxygen species [64]. Vitamin E treatment delayed weight loss in female NP-C mice and resulted in slight improvement in Rota-rod performance [65]. Another antioxidant, ubiquinone (Co-enzyme Q10) is markedly reduced in NP-C patients. However, preliminary results indicate the treatment has little benefit since CoQ10 barely passes the blood–brain barrier [Madra M & Sturley S, Ubiquinone metabolism and Niemann–Pick type C disease (2010), Unpublished Data].

The accumulation of excess cholesterol and glycosphingolipids, including sphingosine, in the acidic compartment of NP-C cells may be caused by the depletion of calcium. Many molecules can increase subcellular calcium levels; for example, curcumin, the active ingredient in the spice turmeric, is both effective and nontoxic and increases survivorship in NP-C mice [66].

Conclusions

Although no cure yet exists, the treatment of NP-C disease has made significant strides in the past decade, from the earliest treatments with statins to the latest trials with sugars, such as miglustat and cyclodextrin (Figure 2). Along the way we have learned much about the transport mechanisms, toxic lipids and correlations to other diseases. Clinically, the sole treatment is miglustat, stabilizing patients for several years with some inhibition of symptom progression, thus at least buying time for this disease. A single, early dose of cyclodextrin has remarkable effects in the NP-C mouse model, by persistently alleviating the lysosomal–endoplasmic reticulum blockade and thus significantly extending lifespan. The mechanism remains elusive and the extension of CYCLO treatment to humans is difficult since 7-day treatment in a mouse would represent an *in utero* intervention in humans. Moreover, cyclodextrins have also been shown to promote the toxic aggregations of amyloid- β peptide, a characteristic of AD, suggesting caution for potential human treatments [67]. These treatments and others underline the importance of studying this disease from multiple angles. Not only do we need to elucidate the transport defect, but we need to repair the neuronal damage. In the approximate 12 years since isolation of the first disease-causing gene, we have seen the NP-C research field move from focusing on the consequences of cholesterol, to pursuing the role of other lipids, thus opening a variety of new treatment options. Indeed, combination treatments (e.g., miglustat, cyclodextrin and/or

allopregnanalone), perhaps acting synergistically, have yielded the greatest increase in survivorship in NP-C animals [43] and show the greatest promise for treating patients.

Future perspective

The challenge for the future of NP-C disease is to translate the advances of the past decade into an effective therapy. Direct treatments of drugable targets within NP-C-related signaling pathways hold a key role in future therapies. However, until the complete pathogenesis, function, targets and related pathways of the NP-C proteins are understood, a multidrug treatment regime will likely be maintained for patients to ensure optimal suppression of symptoms. The vast number of drug strategies that currently exist and were discussed in this review will undoubtedly open further avenues of research. Ultimately, we must find a way to replace faulty proteins and lost neurons and thus reverse the severe damage that already exists in patients at diagnosis.

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Figure 1. Therapeutic intervention for Niemann–Pick type C disease must target the neuron

The complex nature of Niemann–Pick type C (NP-C) disease involves the accumulation of a variety of toxic substrates in the E/L compartment. Administration of cyclodextrin and miglustat and overexpression of Rab9 have effectively targeted two key metabolites – cholesterol and sphingolipids (shown). LXR agonists, such as T0901317, elevate cholesterol excretion from the brain. GSK3 β causes hyperphosphorylation of the tau protein, inducing the formation of neurofibrillary tangles and can be inhibited by lithium. CDK5 overexpression is associated with neurodegenerative pathways and its inhibition with drugs, such as R-roscovitine, reverses these effects. Curcumin allows for elevation of calcium within the acidic compartment of the cell reducing lipid accumulation. Neurosteroids, deficient in NP-C mice, act at the neuron synapse to relay signals, allopregnanolone potentially replaces this insufficiency [34,41,43,51,56,58,67,68].

E/L: Endosomal/lysosomal; LXR: Liver X receptor.

Figure 2. Impact of current treatment strategies in the murine model of Niemann–Pick type C disease

NB-DNJ blocks the production and build-up of gangliosides by inhibiting glycosyltransferase and is the only approved treatment in humans for NP-C and Gaucher's disease under the name Miglustat. In early studies it demonstrated an approximately 20% improvement; however, subsequent studies have demonstrated greater efficacy. The anti-inflammatory agent, ibuprofen increased longevity in mice either alone or in combination with miglustat. The increase in calcium in the acidic compartment of the cell and, consequently, the reduction in the accumulation of lipid species by curcumin also increases lifespan by 35%. The upregulation of Rab9 could also be a potential therapy for this disease, because upregulation in a transgenic mouse model also increases lifespan by 22%. Of the singular therapies, the best treatment to date in the murine system is the cholesterol chelator, CD. It achieves a remarkable improvement in lifespan, of almost 46%. To date, combination treatments, perhaps acting synergistically, yield the greatest increase in survivorship in NP-C animals, with the highest increase nearing 50% (miglustat, CD and allopregnenalone). Ultimately it is the replenishing of Purkinje neurons that will be required; to this end, stem cell therapy provided a 20% improvement in lifespan of the diseased mouse. The data shown were extracted from the following publications, indicated **a–h** [34,41,43,45,50,56,61,65]. CD: Cyclodextrin.

Table 1

Toxic metabolic accumulations in Niemann–Pick type C disease.

Organism	Toxic metabolite		Ref.
	Brain	Liver	
<i>Homo sapiens</i>	Cholesterol, sphingomyelin, sphingosine, lactosylceramide, glucosylceramide, GM2 gangliosides, GM3 gangliosides, neurofibrillary tangles and hyperphosphorylated tau	Cholesterol, sphingosine, bis(monoacylglycerol) phosphate and glycosphingolipids	[22,69–71]
<i>Mus musculus</i>	Cholesterol, GM2 gangliosides, GM3 gangliosides, neutral glycolipids and hyperphosphorylated tau	Cholesterol, phospholipid (sphingomyelin) and sphingosine	[22,45,72–74]
<i>Felis catus</i>	GM2 gangliosides and GM3 gangliosides	Cholesterol, lactosylceramide, glucosylceramide and phospholipid	[22,74]

Niemann–Pick type C disease is a lysosomal storage disorder and it is hypothesized that the malfunctioning of this organelle arises from one or more toxic metabolites.