

New Agents and Approaches to Treatment in Niemann-Pick Type C Disease

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Abstract: Niemann-Pick disease type C is an autosomal recessive disorder caused by mutations in either one of the two genes, *NPC1* or *NPC2*, which encode proteins involved in the regulation of normal transport and/or processing of free cholesterol. Several types of lipids including free cholesterol (unesterified), sphingosine, sphingomyelin, phospholipids and glycosphingolipids (glucosylceramide and gangliosides GM2 and GM3) are accumulated in lysosomes and late endosomes of cells, with pronounced concentrations in the liver and the spleen. The key laboratory diagnostic test for NP-C is filliping staining of cultured skin fibroblasts from the patient, to demonstrate free cholesterol accumulation in lysosomes secondary to impaired intracellular cholesterol transport. The symptomatology and rate of disease progression are strongly influenced by age at disease onset and different clinical forms have been described on this basis: Perinatal, Early-infantile (EI), late-infantile (LI), juvenile and adult forms. Clinical symptoms include progressive neurological deterioration and visceral organomegaly. Nowadays there is no fully effective treatment, only supportive measures for relief of specific manifestations of the disease. The intervention to slow disease progression is the most promising therapy. A number of experimental disease – specific therapies, based on the molecular pathology of NP-C, have been tested in cell culture and animal models including neurosteroids, cholesterol – binding agents, curcumin and Miglustat. This paper summarizes the recent developments that have been investigated for the treatment in patients and animal models with NPC. Current therapeutic approaches have been classified based on the targeting of cellular function, the anti – apoptotic cellular mechanisms and the stem cells therapy.

Keywords: Cyclodextrins, curcumine, miglustat , neurosteroids, niemann-pick disease type C, therapeutic approaches.

1. INTRODUCTION

Niemann-Pick disease type C (NP - C) is a neurovisceral lysosomal lipid storage disease characterized by progressive neurological deterioration. NP – C is an autosomal recessive disorder caused by mutations in either one of the two genes, *NPC1* or *NPC2*, which encode proteins involved in the regulation of normal intracellular trafficking through sequential activities within a common pathway [1,2]. Despite extensive investigations, the specific functions of these proteins are not well understood, although functional forms of both proteins are required for normal lipid transport since a deficiency in either one leads to the NPC phenotype. In 1989 was found that the sphingomyelinase deficiency could be corrected in fibroblasts from Niemann-Pick type C patients by removal of the lipoprotein fraction from the culture medium [3]. This is consistent with the view that the primary defect is one affecting the cellular transport and/or processing of free cholesterol and that it is the intracellular storage of cholesterol that causes a marked attenuation of lysosomal sphingomyelinase activity. Several types of lipids including free cholesterol (unesterified), sphingosine, sphingomyelin, phospholipids and glycosphingolipids (glucosylceramide and gangliosides GM2 and GM3) are accumulated in lysosomes and late endosomes of cells, with pronounced concentrations in the liver

and the spleen. In the liver and spleen, excess storage of unesterified cholesterol, sphingomyelin, glycosphingolipids and sphingosine can lead to visceral symptoms such as organomegaly and liver dysfunction, while increased levels of glucosylceramide, lactosylceramide, and gangliosides in the brain could contribute to the neurological manifestations of the disease [4]. Normally, lipids are transported from lysosomes to the endoplasmic reticulum (ER) and the plasma membrane. In NPC, this transport is disrupted, with the consequent late endosomal - lysosomal lipid storage [5,6].

The key laboratory diagnostic test for NP-C is filliping staining of cultured skin fibroblasts from the patient, to demonstrate free cholesterol accumulation in lysosomes secondary to impaired intracellular cholesterol transport. Evaluation of the rate of intracellular cholesterol esterification is a useful complementary test. Molecular genetic testing for *NPC1* and *NPC2* gene mutations confirm the diagnosis in patients with a variant biochemical phenotype, as well as enable early and reliable prenatal diagnosis [7].

NP-C has a highly variable clinical presentation. The symptomatology and rate of disease progression are strongly influenced by age at disease onset and different clinical forms have been described on this basis: perinatal, early-infantile (EI), late-infantile (LI), juvenile and adult forms. Clinical symptoms include progressive neurological deterioration and visceral organomegaly. Neurodegeneration begins with clumsiness and progressive ataxia followed by a range of symptoms that can generally include dysmetria, vertical

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supranuclear ophthalmoplegia, cataplexy, seizures, dystonia, pyramidal signs, dysphagia and dementia [8].

There is no fully effective treatment, only supportive measures for relief of specific manifestations of the disease. The investigation of possible treatments for NPC has been the subject of several reviews [9,10]. A number of experimental disease – specific therapies, based on the molecular pathology of NP-C, have been tested in cell culture and animal models. These include neurosteroids and cholesterol – binding agents [11]. Recently, curcumin has been suggested to have beneficial effects on intracellular calcium homeostasis and lipid metabolism in NPC1 mutant mice [12]. Miglustat (Zavesca®; Actelion Pharmaceuticals Ltd, Allschwil, Switzerland) is the first and only approved therapy for patients with NPC. Miglustat is approved in the European Union (EU), USA, Canada, Brazil, Australia, Turkey, Israel, Switzerland, South Korea and New Zealand for the treatment of patients with Gaucher disease. In January 2009 the EU Commission extended miglustat's indication to include the treatment of progressive neurological manifestations in adult and paediatric patients with NP-C. This was followed by authorization in Brazil and South Korea [10]. The gene replacement or repair is not yet practicable for NPC and related disorders. The intervention to slow disease progression is the most promising therapy. A short – term goal in this field is to find a small molecule treatment by these approaches, whereas a longer – term goal is to develop a curative treatment based upon molecular biological methods such as gene replacement therapy. The ideal treatment would prevent cell death and maintain normal brain function. This paper summarizes the recent developments that have been investigated for the treatment in patients and animal models with NPC. Current therapeutic approaches have been classified based on the targeting of cellular function, the anti – apoptotic cellular mechanisms and the stem cells therapy.

2. STEROL PATHWAY TARGETING

2.1. Cholesterol-Lowering Drugs

In the earlier years of NPC research, 25 patients with NPC were treated with the combination of lovastatin, cholestyramine and nicotinic acid, as well as dimethyl sulfoxide (DMSO). The first three drugs, but not DMSO, either individually or in combination, lowered liver and plasma cholesterol levels [13]. A mouse model with a disrupted *Npc1* gene was used to study 2 cholesterol-lowering drugs (nifedipine and probucol) and the effects of introducing a null mutation in the low density lipoprotein receptor. Although these treatments significantly ameliorated liver cholesterol storage, there was little effect on the onset of neurological symptoms [14]. The treatment of patients with NPC with drugs that lower somatic cholesterol has not had significant effects on the neurological symptoms. Likewise, controlled diets to reduce cholesterol have not been successful [9].

2.2. Cyclodextrins

The HPBCDs (hydroxypropyl – β – cyclodextrin) are cyclic oligosaccharides consisting of seven β - (1-4) glucopyranose units. The ring of sugar units creates with a hydrophobic interior that has been shown to have a very high affinity for sterols. Authors have reported that intraperitoneal

delivery of cholesterol – mobilising cyclodextrins (2 – hydroxypropyl – β – cyclodextrin) with probucol or nifedipine (which have previously been shown to lower liver cholesterol animal model) decreased liver storage of unesterified cholesterol without altering the depressed levels of esterified cholesterol in *Npc1* mice. However, intraperitoneal or intrathecal delivery of cyclodextrins only slightly delayed the onset of neurological symptoms. This effect on neurological symptom may be partially due to their apparent non-permeation of the blood brain barrier [15]. When a single dose of 2 – hydroxypropyl – β – cyclodextrin is given to NPC mice at an age of 7 days (prenatal development in humans and more permeable blood brain barrier in mice), lysosomal concentrations of free cholesterol are lowered, esterified cholesterol is increased and cholesterol synthesis is reduced along with corresponding effects on the cholesterol regulatory pathway and decreased expression of inflammatory factors in the liver and brain [16]. A combination treatment trial using N-butyldeoxyjirimycin (NB-DNJ) and allopregnanolone increased lifespan for *Npc 1* mice receiving only 2-hydroxypropyl- β -cyclodextrin, the vehicle for allopregnanolone. The administration of 2-hydroxypropyl- β -cyclodextrin alone, but with greater frequency, might provide additional benefit [17].

At present, HPBCD represents a tool for exploring the cell biology of NPC disease that needs further experimental validation in animal models before it is considered as a therapeutic agent.

2.3. Neurosteroids

Neurosteroids are steroids synthesized *de novo* in the brain, or converted into neuroactive steroids in the brain, from steroids derived from the circulation. All steroids and neurosteroids are synthesized from cholesterol through the participation and concerted action of a series of steroidogenic enzymes. Langmade *et al.* [18] noted that the failure to properly traffic lipoprotein cholesterol in NPC1 results in impaired oxysterol and steroid synthesis. The treatment of *Npc1* *-/-* mice with the neurosteroid allopregnanolone and a synthetic oxysterol ligand (T0901317), delayed the onset of neurologic symptoms and prolonged life span, suggesting that the treatment bypassed the cholesterol trafficking defect. The therapy preserved Purkinje cells, suppressed cerebellar expression of microglial-associated genes, and reduced infiltration of microglia in cerebellar tissue. Transfection assays correlated the efficacy of treatment with activation of murine pregnane X receptor (PXR) *in vivo* [18].

The allopregnanolone (ALLO) is a neurosteroid required for normal neuronal growth and survival and brain development but it is shown deficient in the central nervous system (CNS) of *Npc 1* mice due to age - correlated decreases in expression of enzymes of neurosteroid biosynthetic pathways [19]. Allopregnanolone has been shown to reduce levels of reactive oxygen species, lipid peroxidation, and peroxide-induced apoptosis in human NPC fibroblasts or NPC1 knockdown cells, which suggests correction of an abnormal cellular redox state as a therapeutic strategy. A single dose of allopregnanolone in the neonatal period to NPC mice resulted in a doubling of life span, substantial delay in onset of neurological symptoms, survival of cerebellar Purkinje and

granule cell neurons, and reduction in cholesterol and ganglioside accumulation. The mechanism by which allopregnanolone elicited these effects is unknown [11]. Administration of allopregnanolone solubilized in 2 hydroxypropyl- β -cyclodextrin (HPBCD) to Npc1 mice at postnatal day 7 (P7) was reported to be beneficial, with treated mice exhibiting delayed clinical onset, an increase of average lifespan to approximately 120 days compared to 84 days for untreated mice, and reduced ganglioside accumulation [16]. It has been demonstrated for the first time that a single injection of ALLO at postnatal day 7 significantly reduced cholesterol accumulation, in both neurons and microglia, in several brain regions of NPC1 mice. ALLO treatment also reduced abnormal autophagic activity as well as lysosomal dysfunction in neurons and in microglia and reduced microglial reaction. The neurosteroid treatment improved myelination in brains of Npc 1 mice in a cholesterol accumulation-independent manner, suggesting that ALLO treatment may activate multiple cell signalling pathways [20].

3. SPHINGOLIPID PATHWAY TARGETING

3.1. Substrate Reduction Therapy

Substrate reduction therapy utilizes drugs that reduce synthesis of metabolic precursors or products which themselves are known to accumulate in storage diseases. Based on the knowledge that sphingolipids are major components of the abnormal lysosomal lipid accumulation that occurs in NPC, many potential therapeutic strategies have been directed at the regulatory pathways for this lipid class. Miglustat (N-butyldeoxynojirimycin; NB-DNJ; OGT-918) is a small iminosugar molecule that reversibly inhibits glucosylceramide synthase, the enzyme that catalyses the first committed step in glycosphingolipid synthesis [21]. Evidence suggests that Miglustat might also have beneficial effects on pathogenetic NP-C cellular pathways associated with calcium homeostasis [12].

Only agents that appear to cross the blood brain barrier alleviate neurodegeneration and extend lifespan. The ability of miglustat to cross the blood brain barrier indicated its potential use as a therapy for lysosomal storage diseases affecting the central nervous system.

In 2001 Zervas and colleagues demonstrated that daily administration of an inhibitor of glucosphingolipids (GSL) synthesis, N-butyldeoxynojirimycin or Miglustat to NPC 1 mice resulted in a reduction accumulation of GSLs, a delay in onset of clinical signs, and a 30% increase in lifespan [22]. In an adult patient with NPC was shown that depletion of glycosphingolipids by Miglustat treatment reduced pathological lipid storage, improved endosomal uptake and normalised lipid trafficking in peripheral blood B lymphocytes. The glycosphingolipid accumulation, rather than cholesterol storage, is also the primary pathogenetic event in NPC [23]. Pivotal efficacy data were reported from a 12-month randomized, controlled, clinical trial involving 29 juvenile and adult patients, and a parallel, non-controlled sub-study, involving 12 patients aged 4–12 years. The primary study end point – horizontal saccadic eye movement velocity (HSEM- α) was improved with Miglustat versus standard care in adult and juvenile patients; similar improvements were seen in children included in the paediatric sub-study. Improved

swallowing capacity, stable auditory acuity, and slower deterioration of ambulation were also seen in Miglustat-treated patients aged over 12 years [24]. Brain magnetic resonance spectroscopy was used to assess the effects of 24 months' Miglustat treatment in three adult patients with NPC [25].

A disease stability analysis of 19 adult or juvenile patients who completed at least 12 months of Miglustat therapy, based on four key parameters of disease progression (HSEM- α , swallowing, ambulation and cognition), showed that 68% had stable disease after treatment [10].

Further data, indicating stabilization of key parameters of neurological disease progression in NP-C, were reported in a retrospective observational cohort study in 66 patients with a mean (standard deviation) age of 9.7 (7.6 years) [26].

The efficacy of Miglustat in patients with NP-C has also been demonstrated in a number of case series. In two male Taiwanese patients, who started Miglustat therapy aged 14 and 9 years, were observed substantial improvements in swallowing and ambulation by month 6 of treatment, followed by stabilization of neurological symptoms between months 6 and 12 [25]. Santos *et al.* studied the effects of Miglustat treatment in a 9 year old Brazilian patient for 12 months, reporting a rapid and positive impact of therapy on cognitive function, ataxia, dysarthria and ophtalmoplegia [28]. In addition, functional disability (assessed on the disability scale published by Iturriaga *et al.*) was reduced [8]. Efficacy data from paediatric patients who received Miglustat for up to 24 months and analysis of key markers of neurological disease progression in patients treated for at least 12 months have been reported to assess the effect of Miglustat on disease stability [29]. Pineda *et al.* have evaluated the efficacy and tolerability of Miglustat in 16 symptomatic NP-C patients, with comparative reference to one neurologically asymptomatic untreated patient, for several years. All patients were assessed using a standardized protocol: disability and cognitive function scales, positron emission tomography (PET) and biochemical markers. Miglustat was generally well tolerated. Miglustat appeared to stabilize neurological status in juvenile-onset NP-C patients, but therapeutic benefits appeared smaller among younger patients who were at a more advanced stage of the disease at baseline [30].

Based on findings from a prospective clinical trial, pre-clinical and retrospective studies and case reports, the pharmacology, efficacy, safety and tolerability of Miglustat in patients with NP-C have been recently reviewed [10]. Miglustat was generally well tolerated in treated patients. Serious adverse events have not been reported. At the onset of treatment, some patients have experienced episodes of diarrhoea and flatulence which have been managed satisfactorily using proper dietary and nutritional care such as the "bland diet", an oral re-hydration solution, combined restriction of lactose, saccharose and loperamide [30]. Occasionally have been reported cognitive symptoms as lethargy, memory impairment and depression in one paediatric patient during one year of treatment [24]. Another patient initially suffered from insomnia, fine tremors and weight loss in the absence of gastrointestinal disturbances during the first month of treatment and within four months the weight had returned to normal, the fine tremors had disappeared and the insomnia had diminished [28].

3.2. Curcumin

Lloyd-Evans *et al.* found that lysosomal sphingosine storage and reduced lysosomal calcium levels were early events in development of the NPC phenotype in normal human cells exposed to the NPC-inducing drug U18666A [12]. In this model, accumulation of cholesterol, sphingomyelin, and glycosphingolipid was a secondary event. Pharmacologic elevation of cytosolic calcium or reduction of sphingosine content reversed the NPC phenotype in several cellular models of NPC, and sphingosine alone induced the abnormal calcium phenotype in a concentration-dependent manner.

Treatment of *Npc1*^{-/-} mice with curcumin elevates cytosolic calcium levels, increased life expectancy by 35% and slowed the rate of disease progression by 3 weeks. The authors concluded that NPC1 is involved in sphingosine efflux from lysosomes, and that lysosomal sphingosine accumulation in NPC alters intracellular calcium concentration and causes abnormal endocytic trafficking.

4. APOPTOSIS INHIBITORS AND STEM CELLS

Studies of the neurodegenerative effects in NPC have suggested possible therapeutic roles for anti-apoptotic agents. Upon treatment with c-Abl inhibitor imatinib, the mice exhibit improved Purkinje cell survival, reduced apoptosis in the brain, reduced neurological symptoms, improved body movement and longer life span [31].

In order to evaluate the phenotypic effects of implanted neural stem cells (NSCs) in the mouse model of Niemann-Pick C (NPC) disease, Ahmad *et al.* injected a well-characterized clone of murine NSCs into the cerebella of neonatal *Npc1*^{-/-} and control mice. The implanted cells survived and were abundant in some regions of the cerebellum. Life span was lengthened in NPC mice with the implanted NSCs. However, the rate of weight gain and subsequent weight loss, resulting from neurodegeneration, was not significantly different from un-injected controls. Thus, in this small group of NPC mice, a single administration in the neonatal period of the NSCs was only partially therapeutic [32].

5. CURRENT CLINICAL MANAGEMENT

Supportive therapies are variably effective for the alleviation of clinical manifestations of NP-C and can improve patient's quality of life. Pharmacological treatments for seizures, cataplexy, dystonia, and spasticity can be employed successfully in patients with NPC. Dietary manipulation including softening and thickening of foods is a useful measure, but most patients eventually require gastrostomy tube placement to maintain adequate fluid and caloric intake. Cataplexy is well recognized as a manifestation of NPC that can be effectively managed with Clorimipramine. Dystonia and tremor respond well to anticholinergic drugs and botulinum toxin can also be effective in selected cases. Gastrointestinal signs are often seen in NP-C patients, and diarrhoea can be frequent in treated and non – treated patients. Loperamide and dietary modifications can be used. Seating and general nursing care are important considerations, as is the management of secretions in the respiratory tract. The com-

bination of physical therapy, bronchodilators and prophylactic antibiotics sometimes has been used.

CONCLUSIONS AND FUTURE PROSPECT

There is no demonstrated therapy providing an effective, long- term treatment and rescue of the NPC phenotype. Compounds such as Miglustat, allopregalalone, oxysterols and cyclodextrins show promise in slowing the progress of the disorder, but these and other compounds are still far from providing the type of treatment, let alone a cure, that is desired for this devastating disease. If therapeutic targets were to be identified, then the power of modern rational drug design could be applied for NPC.

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