Chapter 4

Use of 2-Hydroxypropyl-Beta-Cyclodextrin for Niemann-Pick Type C Disease

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Abstract

Niemann-Pick disease type C (NPD-C) is a rare neurodegenerative disorder characterized by a lysosomal storage disorder. Treatment has been supportive and symptomatic. In animal studies, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) showed a significant decrease in cerebellar damage, neurological progression, and higher lifespan. Based on these results, HP-β-CD has been tested in NPD-C patients for last 8 years. The first compassionate uses of intravenous HP-β-CD obtained a limited improvement in neurological symptoms, probably associated to the non-permeation of the blood-brain barrier. The change or combination with intrathecal administrations of HP-β-CD achieved higher benefits, especially improvement or stabilization of NPD-C progression. Biomarkers of neurological cholesterol homeostasis are being investigated in order to quantify the response of HP-β-CD treatment. The results of a clinical trial recently published have reproduced the slowing of NPD-C progression in 14 patients treated with a dose-escalation protocol of HP-β-CD intrathecal monthly infusions, with respect to a historical comparison cohort. The safety profile of this therapy is acceptable, being the loss of hearing as the most frequent adverse event. However, some severe toxicities have been reported in relation with HP-β-CD, including chemical meningitis and fever. The short experience with HP-β-CD suggested that it could be effective in the management of NPD-C.

Keywords: Niemann-pick type C, 2-hydroxypropyl-β-cyclodextrin, cholesterol, neurodegenerative diseases, blood-brain-barrier

1. Introduction

1.1. Etiology

Niemann-Pick disease type C (NPD-C) is a rare autosomal recessive disorder characterized by lysosomal lipid storage in which faulty intracellular lipid transport leads to accumulation of unesterified cholesterol and glycosphingolipids in several neurovisceral tissues [1, 2].

NPD-C is caused by mutations in either the *NPC1* or *NPC2* genes. The *NPC1* gene, located on the long arm of chromosome 18 (18q11.2), encodes a large (142 kDa) membrane glycoprotein placed in endosomes and lysosomes. This protein mediates intracellular cholesterol trafficking via binding of cholesterol to its N-terminal domain. *NPC2* gene, located on the long arm of chromosome 14 (14q24.3), encodes a small (16 kDa) lysosomal protein that binds to cholesterol. NPC1 and NPC2 proteins seem to act in the cooperative transportation of molecules within cells. Recently, it has been shown, the N-terminal domain of NPC1 protein may interact with NPC2 protein to facilitate cholesterol efflux from the late endosome and lysosomes [3, 4].

The majority of patients show mutations in the *NPC1* gene (95%), whereas a much smaller number suffer mutations in the *NPC2* gene, but the resulting phenotypes are clinically indistinguishable. Loss of function of either of these proteins results in an accumulation of cholesterol and other lipids, including sphingomyelin, sphingosine, and gangliosides (GM2 and GM3), within the endosomes and lysosome [3, 4].

1.2. Diagnosis

The diagnosis of NPD-C requires a combination of clinical, cellular, and molecular criteria. NPD-C is suspected on the basis of the clinical features. Systemic manifestations such as hepatosplenomegaly neonatal, cholestatic jaundice, or splenomegaly can lead to diagnosis, but, due to the heterogeneous clinical phenotype, diagnosis is often delayed for many years or missed altogether [4]. A Suspicion Index tool might be useful as a screen for NPD-C, a risk prediction score ≥70 indicates a strong suspicion for NPD-C [5]. The diagnosis of NPD-C is confirmed by biochemical testing that demonstrates impaired cholesterol esterification and positive filipin staining in cultured fibroblasts obtained from a skin biopsy. Filipin staining demonstrates intense punctate pattern of fluorescence concentrated around the nucleus, consistent with unesterified cholesterol. Molecular genetic testing of *NPC1* and *NPC2* are commercially available and they detect pathogenic variants in approximately 94% of individuals with NPD-C [4].

The filipin assay is unable to provide a firm diagnosis in fibroblasts with "variant" phenotypes that represent one-third of NPD-C cases. Moreover, the assay is invasive and the results can delay for 3 months. Genetic analysis is an important diagnostic tool, though, due to cost considerations, generally applied as a confirmatory rather than screening test. In recent years have been identified promising NPD-C biomarkers. One of these markers, cholestane-3β,5α,6β-triol, which is a cholesterol oxidation product, has emerged as a sensitive diagnostic for NPD-C [6, 7].

1.3. Clinical presentation and symptoms of Niemann-Pick disease

Systemic involvement of liver, spleen, or lung, is present in ≥85% of patients, and precedes the development of neurologic symptoms. The age of onset and clinical presentation of NPD-C is highly variable.

Neonatal and infantile presentations: Occasionally, ultrasound examination in late pregnancy has detected fetal ascites; infants thus identified typically have a severe neonatal liver disease with jaundice and persistent ascites. Infiltration of the lungs with foam cells can be present. Many infants die at this stage. Of children who survive, many have hypotonia and psychomotor retardation whereas others may have complete resolution of symptoms, only to present with neurologic disease many years later [4].

Childhood presentations: These patients typically have cerebellar involvement characterized by clumsiness and gait problems progressing to frank ataxia and slow cognitive deterioration Vertical supranuclear ophthalmoplegia is another early manifestation. Progressive dystonia, dysarthria, and dysphagia occur, eventually impairing oral feeding, and approximately one-third of patients develop seizures [4].

Adolescent and adult presentations: The clinical presentation is similar to childhood onset with ataxia, supranuclear vertical gaze palsy, cognitive impairment, except that progression is generally much slower. Other adults present with cognitive dysfunction or psychiatric disturbances as major depression, schizophrenia, or bipolar disorder [4].

1.4. Previous treatments employed

There is no curative treatment for NPD-C. The disease management is individualized and it consists mainly of symptomatic treatment. Seizures, dystonia, and cataplexy can respond to drugs. Other symptomatic measures like physiotherapy in spasticity, gastrostomy tube placement to prevent aspiration and/or inadequate nutrition in patients with progressive dysphagia, and bronchoalveolar lavage to improve pulmonary function are useful in the disease management. Combination of drug regimens have been shown to lower hepatic and plasma cholesterol but there is no evidence that these results affect the progression of the disease in humans or murine models. Behavioral and speech problems or schooling difficulties should be referred to as psychiatric team and special schooling [8, 9].

To date, miglustat is the only disease-specific drug approved in Europe, Canada, and Japan. The drug works by inhibiting the glucosylceramide synthase enzyme that is responsible for the first step in the synthesis of most glycosphingolipids. Miglustat has shown to stabilize key parameters of neurological disease progression in patients of all ages, but it has no effect on the systemic manifestations or intracellular cholesterol accumulation associated with this disorder [10].

There is a persistent search for new treatments to prevent or slow down the progression of NPD-C. Investigational therapies in course are 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD) or hematopoietic stem cell transplantation.

VTS-270 (Kleptose® HPB, Roquette Pharma, France) and Trappsol® Cyclo™ (CTD Holdings, Inc., Alachua, FL) are HP-β-CD products under investigation as novel treatments for NPD-C. Differences between these two products have been studied based on ion distribution and abundance profiles using mass spectrometry methodology as a means to assess key molecular distinctions between products. Trappsol® Cyclo™ was found to have a higher degree of substitution compared with VTS-270, with a greater number of hydroxypropyl groups and increased levels of dimeric ions. Additional differences in ion mobility profiles were found, there is a much greater level of non-specific chemical "noise" associated with Trappsol® Cyclo™.

These two products are not chemically equivalent and therefore may not be biochemically equivalent or lead to comparable formulations from a clinical development perspective. The data suggest that biological and potential therapeutic equivalence should not be assumed. Further studies are needed to examine potential differences in biological and therapeutic effects of Trappsol® Cyclo™ and VTS-270 [11].

2. Preclinical studies

2.1. *In vitro* experience

The efflux of cholesterol from cells in culture to cyclodextrin acceptors has been reported to be substantially more rapid than efflux induced by other known acceptors of cholesterol. A comparison of the time course of cellular [3H]cholesterol efflux mediated by HDL3 or by various concentrations of cyclodextrins showed the release of 50–90% of L cell [3H]cholesterol after 8 hours of incubation with HP-β-CD and methyl-β-cyclodextrin (M-β-CD) at 10 mM. The order of efficiency in accepting cholesterol was found to be M-β-CD > HP-β-CD > beta-cyclodextrin. The kinetics of the cholesterol efflux time course studies suggested that incubation of L cells with cyclodextrin resulted in the rapid equilibration of labeled cholesterol between cells and medium. [12].

Several studies have shown that cholesterol released from late endosomes/lysosomes of NPC-proteins deficient cells by HP-β-CD reaches the cytosolic compartment and is accessible to the endoplasmic reticulum (ER). In cultured cerebellar neurons, astrocytes, and microglia from NPC1-deficient mice, the sequestered cholesterol was mobilized to the ER by low concentrations (0.1–1.0 mM) of HP-β-CD [13].

In murine models of NPC1, cell culture studies have shown that M-β-CD is more potent in exchanging cholesterol than HP-β-CD. Efficacy comparison of M-β-CD and HP-β-CD in reducing cholesterol accumulation in late endosome/lysosome in human fibroblasts NPC1/NPC2 deficient after treatment with 300 μM M-β-CD and HP-β-CD for 1 day have shown to reduce the cholesterol accumulation detected by filipin labeling. After 2 and 3 days after treatment, cholesterol accumulation started to increase, but there was still a significant reduction. In concordance with animal studies, M-β-CD produces effects equivalent to those of HP-β-CD

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at lower concentrations [14].There are several theories about the mechanism by which cyclodextrins affect cholesterol homeostasis but none have been confirmed.

In vitro beta-cyclodextrins showed a high affinity for sterols as compared to other lipid and, because of the relatively high specificity of this substance for cholesterol, it was suggested that beta-cyclodextrins might be effective in modifying cholesterol metabolism in vivo.

2.2. Animal studies

The unexpected discovery of the utility of cyclodextrin in NPD-C was observed in a study in Npc1−/− mice treated with a combination therapy of two drugs, one of them (allopregnanolone) formulated in a cyclodextrin complex [15]. A later study showed the same cholesterol intraneuronal storage reduction and longevity increase with the combination of allopregnanolone and cyclodextrin than with the control arm, treated only with the cyclodextrin [16]. These results led researchers to perform studies to address the role of cyclodextrin as a possible treatment. The Npc1−/− mice receiving subcutaneous (SC) or intraperitoneal cyclodextrin every other day for 2 weeks revealed a slight decrease of intraneuronal accumulation of either cholesterol or gangliosides. Both routes showed similar outcome, but SC administration seemed to be a slightly more efficacious [16]. In another study, Npc1−/− mice treated with a single SC injection at 7 days of age of HP-β-CD (4000 mg/kg body weight) prolonged the average life (108 days).

Treatment with cyclodextrin improved hepatic dysfunction and decreased neurodegeneration, increasing the number of Purkinje cells surviving at 49 days of age nearly threefold respect to untreated mice [16]. In previous studies, no significant toxicity was observed following the administration of HP-β-CD except for increased macrophage infiltration of the lungs found at post mortem examination [16, 17]. Other studies in Npc1−/− mice showed that 1500 mg/kg HP-BC-D administered weekly caused a decrease in hepatic unesterified cholesterol concentrations without substantial effect on neurological signs The slight effects of the HP-β-CD on neurological symptoms at low doses may be partially due to their apparent nonpermeation of the blood-brain barrier (BBB) [18].

The efficacy of HP-β-CD was also tested in a feline model of NPD-C. Cats affected with NPD-C were first administered the drug at 3 weeks of age, prior to the onset of clinical signs of disease, and continued to receive the drug weekly. Cats were placed into one of five groups: received no HP-β-CD; received a weekly dose of 1000 mg/kg; 4000 mg/kg; 8000 mg/kg body weight HP-β-CD subcutaneously; or 4000 mg/kg brain weight (120 mg for a 30 g brain weight) HP-β-CD intrathecally every 2 weeks. The preliminary data suggested a similar requirement for doses equal to or greater than 4000 mg/kg to positively affect neurological disease. Nevertheless, doses of 4000 mg/kg body weight resulted in an increase in hearing threshold only after repeated dosing and doses of 8000 mg/kg body weight resulted in significant increases in hearing threshold in both normal cats and cats with NPD-C following the administration of a single dose [19].

Studies in mouse models have shown that systemic administration of HP-β-CD, starting in early neonatal life, diminishes unesterified cholesterol accumulation in most organs, slows

disease progression, and extends lifespan. Studies in adult Npc1−/− mice who received four weekly subcutaneous 4000 mg/kg body weight HP-β-CD at 49 days of age showed reduced whole-liver cholesterol content at 77 days. Comparable improvements were seen in other organs, such as spleen, and lifespan was extended [20]. On the whole, preclinical studies in animals showed that young animals respond more favorably, whereas the older ones may benefit less.

Brain uptake of 2-hydroxypropyl-[14 C]-propyl-β-cyclodextrin was determined in Npc+/+ and Npc1−/− mice using two methods: *in situ* brain perfusion and multi-time-point regression analysis flowing intraperitoneal administration. None of the data collected indicated that HP-β-CD enters the brain [21]. Other experiments examining cyclodextrins with regard to permeability using an *in vitro* model of the BBB have indicated that a small percentage of cyclodextrin may be transported across the barrier [22].

Intrathecal (IT) HP-β-CD (120 mg in 0.6 ml saline) every 2 weeks therapy of feline NPD-C delayed the clinical manifestations of neurological disease, but had no effect on hepatic or pulmonary disease. IT-treated cats showed amelioration of neuronal swelling and axonal spheroid formation in many but not all brain regions, and preservation of Purkinje cell numbers [23]. Research in mouse models of NPD-C also has shown that direct administration of HP-β-CD into the IT or intracerebroventricular (ICV) space at low concentrations has a similar or superior effect on delaying the onset of neurological symptoms as that observed following high systemic doses [24].

Recent researches in NPD-C cats showed that direct administration of HP-β-CD into the cisterna magna prevented the onset of cerebellar dysfunction for greater than a year and reduced in Purkinje cell loss and near normal concentrations of cholesterol and sphingolipids. Cats receiving 1000 mg/kg SC HP-β-CD had a similar occurrence of neurological dysfunction and survival than untreated cats. Nevertheless, cats that received 4000 mg/kg SC HP-β-CD showed modest amelioration of neurological disease and survived age than any untreated cats. Pulmonary toxicity limited the continued dosing of cats in the 8000 mg/kg group. Dose-dependent elevations in mean hearing threshold in cats receiving SC HP-β-CD were observed. In cats receiving intracisternal HP-β-CD, a significant elevation in the auditory threshold was also observed. [25].

3. Experience in humans: first experiences of compassionate use of cyclodextrin

Based on the promising results of preclinical studies in animal models of NPD-C, in November 2008, Dr Hastings applied for individual investigator new drug exemptions (INDs) to the Food and Drug Administration (FDA) for the use of with HP-β-CD in humans. In January 2009, the first NPD-C patient in the world, an Indian child, was treated with HP-β-CD by intravenous (IV) route. Few months later, the first two INDs of HP-β-CD in USA were approved by FDA for two identical twins aged 5-year-old [26]. The "Oakland protocol" of IV HP-β-CD employed in USA was also used in two Brazilian sisters with NPD-C in January 2010 [27].

The orphan designation of HP-β-CD was obtained in May 2010 by FDA [28] and 2 months later by the European Medicines Agency (EMA) [29]. The orphan drug brand Trappsol® Cyclo™ was approved specifically for treating NPD-C. New findings suggested that the passage of HP-β-CD across the BBB was limited [21, 24, 25, 30], and physicians who treated the first patients reported slight benefit with IV route [26]. The FDA approved the request for IT delivery of HP-β-CD in September 2010 [31]. Another orphan drug of HP-β-CD was designated by FDA in February 2013, Kleptose™ (brand VTS-270). Both drugs were being evaluated in clinical trials, moreover compassionate use outside clinical trials has been reported worldwide [32].

Five different approaches have been employed in HP-β-CD therapy:

- IV only.
- IT only.
- ICV only.
- IV at the beginning, and later changed to IT or ICV.
- IV and IT simultaneously.

The IV administration was the first route tested for HP-β-CD in humans. When different reports in animals demonstrated that HP-β-CD administered intravenously cross in very little proportion the BBB [21, 24, 25, 30], this route was changed or combined with IT/ICV administration of the same drug. Theoretically, IT and ICV routes deliver directly the drug in the central nervous system (CNS), the area mainly affected in NPD-C, especially in the neurological symptoms. There is still controversy over whether the IV route contributes in NPD-C therapy. Preclinical reports showed effectiveness in the peripheral manifestations of the disease in organs [20, 25], including the liver, the spleen, and, to a lesser extent, the lungs. However, the influence of HP-β-CD in CNS after IV infusion remains in humans unknown.

4. Experience in humans: efficacy of the intravenous administration of cyclodextrin

The first two patients who received IV HP-β-CD (Trappsol® Cyclo™) in USA were treated since April 2009 with 2500 mg/kg weekly over 8 hours, and later modified to administrations every 2 weeks for convenience. The twins were diagnosed at 3 years old with initial symptoms of hepatosplenomegaly, ataxia and seizure activity, and previously were treated with miglustat, without significant benefits. After 18 months of therapy, any objective improvement was reported, and the disease continued to progress as evidenced by positron emission tomography (PET) imaging and neurological assessments [26]. Both patients were described a transient appearance of slight scattered nodules in lungs during bronchoscopy, which were identified as xanthomas, a deposition of yellowish cholesterol-rich material typical of NPD-C, resolved without treatment changes in IV therapy [32].

In October 2010, the FDA allowed the addition of HP-β-CD IT administration at doses of 350 mg every 2 weeks (initial dose 175 mg/2 weeks) [32, 33]. During the first months of concurrent IV/IT therapy, a significant improvement was observed in the validated NIH NPC Clinical Severity Score (NNCSS), as well as in the brainstem auditory evoked response (BAER) [33]. After 18 months, ICV route was authorized by FDA in the twins through an Ommaya reservoir system. One of them tolerated perfectly the ICV and continues today with the treatment, whereas in her sister, ICV was discarded by insertion bleeding and IT route was recovered. Both patients have experienced improvements in alertness, swallow, head control, and ataxia [32, 34].

The second reported use of IV HP-β-CD (Trappsol® Cyclo™) was in two Brazilian sisters at age of 12 and 16 years old with concomitant miglustat treatment. Dr Vieira employed the same IV protocol with a dose escalation from 1200 to 2500 mg/kg/week. Both patients showed, after 1 year of treatment, objective benefits in PET and NNCSS, as well as improvements in motor dysfunction, cataplexy, psychiatric symptoms, behavior, cognitive functions, and memory [27]. Two later reports informed about the simultaneous treatment with IV weekly and IT twice monthly at least 3 years, maintaining the good results previously reported with IV infusions [35, 36]. No adverse events (AEs) were reported, although the published data were limited and only congress abstracts were reported [27, 35, 36].

Two reported cases of Japanese NPD-C patients diagnosed at 2 months and 13 years were initially treated at 4 and 14 years old with the IV "Oakland protocol", with doses from 80 mg to 2500 mg/kg/three or two times a week, respectively [37, 38]. These were the first published cases of VTS-270 use in NPD-C. The younger patient reported with IV administration, a mild decrease in hepatosplenomegaly and temporary improvements in EEG and stabilization of disability scores the first 6 months. However, rest of the 2 years with only IV infusions a rapid progression of neurological dysfunction and worsening of swallow, speech, rigidity, and seizures were reported [37]. Furthermore, repeated fever with transient diffuse pulmonary cloudiness episodes were observed, an AE probably related to HP-β-CD IV infusion. After 2 years of IV therapy, IT first and later ICV administrations were combined with IV infusions [38]. The combined therapy obtained objective benefits in EEG, PET and magnetic resonance spectroscopy (MRS), and CSF T-tau level reduction. Furthermore, improvements in eye movement, language, and speech were reported, as well as and stabilization of clinical progression for 2 years. No AEs were detected over the 2 years of IV/ICV combined therapy.

The other patient, a girl of 14 years old at the time of HP-β-CD therapy onset, was only treated with IV infusions for 3 years. At the beginning of the treatment the patient showed benefits in EEG, MRS and visual evoked potential (VEP), but after 3 months disappeared. A decrease in hepatosplenomegaly was observed in abdominal ultrasound and body computed tomography (CT). The general condition, neurological tests, and seizure control were stabilized with therapy, reporting only an improvement in alertness. Tolerance of IV therapy was excellent [37].

Drs Hrynkow and Hastings recently reported in a Congress the clinical experience with 11 patients initially treated with IV HP-β-CD more than 6 years [39]. Two of these patients were only treated with IV infusions, whereas in nine patients IT therapy was added later. In the two patients with only IV therapy, one showed a progression in NNCSS similar

AEs associated with administration	AEs recognized as features of NPD-C	Other AEs of interest
Rash	Seizures	Port-a-Cath infection
Generalized rash (trunk, elbow)	Pneumonia	Removal of Ommaya
Tremor/chills/vomiting/fever	Thrombocytopenia	Reservoir
Headache	Viral illnesses	Post-operative delayed Parenchymal hemorrhage
Nausea	Viral syndrome	
Stomach pain		Meningitis

Table 1. Adverse events related to IV with/without IT HP-β-CD administrations.

than previous scores before initiation, whereas the other patient reported a stabilization of NNCSS after IV therapy. In two patients with IV/IT therapy significant decrease in NNCSS were observed comparing pre and post-therapy scores. In the other seven patients was only available post-therapy information, showing in most of the cases an initial de decrease in NNCSS followed with a progressive increase. Other significant improvements were reported, as reduction in hepatomegaly, restoration of language skills, resolution of interstitial lung disease and improvements in fine motor control. The tolerability was favorable, although some AEs were reported during 6 years of therapy (**Table 1**).

Despite the positive results observed in the compassionate use of IV HP-β-CD [26, 27, 32–39], there is no clear evidence to date that IV route has a clinical benefit in CNS symptoms of NPD-C in humans. Most of the reports with neurological improvements combined the use of IV and IT/ICV administrations, making it impossible to demonstrate that these benefits were exclusively associated to IV and not IT/ICV. In addition, doses 1000 times larger than the IT dose should be required to achieve the same concentrations in the CSF reached by IT administration with IV infusions of HP-β-CD. This dosage would probably be unsafe in humans.

5. Experience in humans: efficacy of the intrathecal administration of cyclodextrin

The IT route was proposed when the efficacy of IV infusions of HP-β-CD was questioned. The first cases of IT administrations were combined with IV, as we previously explained [33–37, 39]. At this point, IT route was proposed as the main route for NPD-C therapy. The first published case report of IT HP-β-CD (Trappsol® Cyclo™) administration was a Spanish girl diagnosed and treated at 2 years old. The dosage varied from 200 to 450 mg every 2 weeks. At 38 months an IT Ommaya reservoir (IT-OR) was implanted in order to increase patient convenience and reduce number of punctures, modifying the dosage to 400 mg every 2 weeks. The patient showed improvements in BAER test, whereas neurological symptoms and NNCSS were stabilized. The patient suffered two episodes of seizures with a fever with IT therapy, resolved without any change in treatment and which could be related to HP-β-CD, the procedure or the disease [40].

Dr. Berry-Kravis reported the clinical outcomes of the use of IT HP-β-CD (Trappsol® Cyclo™) in three patients with NPD-C in several congresses [41–43]. Two of them were a couple siblings of 14 and 15 years old who were unable to be enrolled in the Phase 1 clinical trial (NCT01747135) and were treated as a compassionate use. The younger sibling with doses from 200 to 500 mg every 2 weeks obtained improvements in NNCSS, instrumented timed up and go (gait test) and Mullen Scales of Early Learning (cognitive scale), as well as benefits in cognitive functions, seizures, swallow, language and speech [40–43]. The other sibling received doses from 200 to 600 mg every 2 weeks but the benefit was poor, with stabilization of NNCSS, CFS reduction of lysozyme and improvement in cognitive functions [40–43]. After the first three IT infusions were reported post lumbar puncture headache and vomiting in both siblings, but it was not related to the drug and disappeared after a switch to use of Whitacre spinal needles. The clinical information regarding the third patient was insufficient to evaluate the efficacy, although an improvement in balance and gait was reported after 1 year treated with doses from 200 to 400 mg every 2 weeks [43].

The use of IT HP-β-CD with a fixed dose of 200 mg every 2 weeks was reported by Maarup et al. in a single case report. After 18 months of the therapy, the boy showed an improvement in vertical gaze (eye movement) and the consequent decrease in the NNCSS. This study also reported an increase in 24-OH cholesterol, a biomarker of cholesterol redistribution in CNS. On the other hand, an AE was associated to HP-β-CD administration, the well-known hearing loss to high frequency [44].

The use of IT-OR HP-β-CD in two Spanish boys diagnosed of NPD-C at 6 and 10 years old was briefly reported in congresses [45, 46]. The first patient was treated at 11 years with doses from 125 to 525 mg (Trappsol® Cyclo™) every 2 weeks for at least 37 months. Benefits in muscle tone and a decrease in seizures frequency were observed with IT-OR therapy. Initially was reported an improvement in BAER test, although it was alternated with auditory deteriorations [32, 45]. In the other case report, a 16 years patient received IT-OR fixed-doses of 350 mg every 2 weeks for 20 months. The objectives results included BAER tests and NNCSS. Furthermore, improvements in language and speech, ataxia and quality of life were obtained. The most relevant toxicities were intermittent fever and a suspicion of chemical meningitis [32, 46].

A recently published article described the IT therapy of a young NPD-C girl of 22 months [47]. The dosage of HP-β-CD was 175–325 mg every 2 weeks for 20 months. The treatment only achieved improvements in visual contact and motor function with the first doses, as well as slight retardation of disease progression and in the NPD-C disability scale during the first year. After the first year, MRI showed a progression of cerebral atrophy, which was consistent with a clinical disease progression (epilepsy, dysphagia, and worsening motor function). Despite the initial response and the absence of AEs, the IT HP-β-CD was discontinued after 20 months by lack of efficacy.

The employment of HP-β-CD in adult-onset NPD-C has been described with variable results in two publications [48, 49]. Sakiyama et al. reported the IT treatment (VTS-270) of two adult patients of 37 and 28 years with doses from 100 to 400 mg every month. The older patient showed better eye movement and neurological stabilization, whereas the younger patient

reported improvement in NPD-C scales, balance and gait, language and speech, and swallow. In addition, reductions in oxysterol serum concentrations were observed in both patients, a sterol storage biomarker. No AEs were reported in these patients [32, 48]. The worse outcomes observed in two cases reported by García-Robles et al. could be related to the age (49 and 39 years old) and advanced disease at the HP-β-CD onset. The dosages of IT HP-β-CD (Trappsol® Cyclo™) ranged from 175 to 700 mg and 50 to 875 mg every 2 weeks, also using IT-OR route in the second patient. Any objective or subjective improvements were reported in both patients. The older patient received only four doses with optimal tolerance, but HP-β-CD therapy was discontinued when neuropsychiatric symptoms progressed. The other patient suffered two episodes of toxic meningitis as well as worsening respiratory symptoms and swallow. After second chemical meningitis and neurologic progression of the disease, HP-β-CD treatment was discontinued [49].

6. Experience in humans: ongoing clinical trials

Four clinical trials using cyclodextrin for the treatment of NPD-C have been found. One of them has been completed and their results have been published [50] and three are currently ongoing and no preliminary results have been yet published.

- Intrathecal 2-hydroxypropyl-β-cyclodextrin (VTS-270) for Niemann-Pick type C1 (NPC-1) disease. A non-randomized, open-label, Phase 1–2 trial. See ClinicalTrials.gov Identifier: NCT01747135 [50].

Phase 1–2, non-randomized, open-label, study, to assess the tolerability, safety, feasibility, and PK of HP-β-CD administered IT monthly via lumbar injection to drug naive cohorts of NPC-1 patients at doses of 50 mg escalated to a maximum of 1200 mg. The objective is to determine an active dose of HP-β-CD as measured by changes in plasma 24-(S)-hydroxycholesterol (24(S)-HC) concentration and to evaluate the use of biomarkers and potential clinical outcomes of NPC-1. NNCSS is used to assess clinical efficacy. The decision to dose-escalate is based on safety and biochemical data. Safety is assessed by the appearance of AEs with performance of clinical laboratory tests, physical examinations, and with special attention to audiological evaluation. Biochemical efficacy is measured by change from baseline in plasma 24(S)-HC. The PK analysis is assessed for plasma HP-β-CD concentrations. This is the only clinical trials of HP-β-CD with published results.

Eligible patients were aged 2–25 years and had NPC-1 with neurological manifestations. Fourteen patients were enrolled from National Institutes of Health (NIH-cohort). Cohort size was three participants for initial IT doses of 50, 200, 300, 400, and 900 mg (only two patients) administrated IT every month. Three participants were initially dosed with 50 mg ICV via an Ommaya reservoir approximately 6 months prior to initiation of the IT trial. Use of the Ommaya reservoirs was discontinued due to *P. acnes* infection/colonization in two subjects. Due to this problem, initial protocol was amended and ICV route was changed by IT. After initial dosing at the specified cohort dose, participants were dose-escalated based on tolerance and safety data.

As comparison control, a cohort of NPD-C subjects from Natural History study with longitudinal assessments was employed. These patients were not on HP-β-CD treatment. To explore a scheme every 2 weeks, three additional subjects were recruited with the same criteria mentioned above in Rush University Medical Center (RUMC-cohort).

The primary outcome was changed in 24[S]-HC area under the curve ($AUC_{8–72}$) response to drug administration compared with the response after saline administration. The $AUC_{8–72}$ of plasma 24(S)-HC concentrations were established after its determinations at pre-dose, 8, 24, 30, 48, and 72 hours post-dose after either HP-β-CD of saline infusion.

As a secondary objective, NNCSS was used to assess clinical efficacy. Audiological assessments were obtained monthly before each infusion. Also the concentrations of fatty acid binding protein 3 (FABP3) and calbindin D in cerebrospinal fluid (CSF) were assayed.

Finally, 14 NIH-patients and 3 RUMC-patients were enrolled. Twenty-one patients with similar characteristics were identified from the historical database for comparing. For primary outcome (change in 24(S)-HC $AUC_{8–72}$), 121 of 155 post-drug plasma value were greater than post-saline values. Despite the variability, the data suggest a dose-response relationship. All the patients of the study had either FABP3 or calbindin D, a significant negative linear regression slope (only one patient had a significant increase in calbindin D slope).

Regarding clinical efficacy, the total NNCSS for NIH-cohort increased at a slower rate than comparison cohort. These data show a significant reduction in disease progression in the cohort of HP-β-CD treated patients. In a secondary responder analysis, cohorts of treated and comparison subjects were classified as responders when their NNCSS minus hearing was stable or improved. Seven of 14 NIH-cohort subjects were classified as responders, 3 of 3 RUMC-cohort subjects were classified as responders, and none of 21 patients of comparison cohort were classified as responders. Safety will be discussed in Section 7.

The added value of this study was to provide a neurological disease progression comparison among a cohort of NPD-C subjects treated with IT HP-β-CD and a control cohort of NPD-C subjects from Natural History study, indicating a decrease rate of neurological disease progression in the treated cohort. Moreover, this study provides information on the safety of IT administered HP-β-CD and the measurement of biomarkers provided additional support for decreased neuronal damage and improved neuronal cholesterol homeostasis.

- A Phase 2b/3 prospective, randomized, double-blind, sham-controlled trial of VTS-270 (HP-β-CD) in subjects with neurologic manifestations of Niemann-Pick type C1 (NPC-1) disease. (See ClinicalTrials.gov identifier: NCT02534844 and EudraCT Number: 2015-002548-15).

Multicenter, multinational, prospective, randomized, double-blind, sham-controlled, three-part, efficacy and safety trial of HP-β-CD, administered by the lumbar IT route every 2 weeks, with a planned enrollment of approximately 51 subjects with NPC-1 disease. This study is ongoing, but not recruiting participants (male or female subjects, aged 4–21 years of age at time of screening with onset of neurological symptoms prior to 15 years of age).

This study has three parts with different objectives. The objective of Part A is to select the dose of HP-β-CD to be used in Part B and Part C. Three different HP-β-CD lumbar IT doses

(900, 1200, and 1800 mg) will be administered IT every 2 weeks for 8 weeks and 2 weeks for observation in 9 subjects; 3 subjects will receive sham treatment. The criteria for dose selection include safety and tolerability including a thorough audiological evaluation.

The objective of Part B is to evaluate, in a double-blind sham-controlled design, the progression of the neurologic manifestations of NPC-1 disease based on changes in the composite efficacy outcome (consisting of four components of the NNCSS: ambulation, fine motor skills, cognition, and swallowing), after 52 weeks of treatment in comparison to baseline. Part B will evaluate the safety and efficacy of the dose selected from Part A compared to sham control in 51 subjects (randomized 2:1), including the 12 subjects from Part A.

The objective of Part C is to evaluate the long-term safety, tolerability, and efficacy of the dose selected for Part B. This part is an open-label extension with IT treatment every 2 weeks to subjects who either complete Part B or are subjects in Part B who have met rescue therapy criteria. Additionally, subjects who are currently active in the NIH-sponsored Phase 1 protocol (NCT01747135, see above) will also be eligible to participate upon completion of their participation in the Phase 1 study. In this part, subjects will receive treatment until licensed product or end of the program.

The primary outcome measure of the study is NNCSS with a time Frame of 52 weeks. Data for NPD-C score rating will be provided to a centralized independent blinded rater, who will analyze all NPD-C information for all subjects and assign the NNCSS rate. As secondary outcome measures are: clinician and caregiver clinical global impression of change, time to get up and go test, 9-hole peg test, percentage of patients with clinical worsening, and European Quality of Life-5 dimensions quality of life rating (EQ-5D QoL). Moreover, CSF and plasma biomarkers will be measured.

The design enables a selection of best dose based on efficacy, safety, and tolerability according to an evaluation by a Committee. The dose chosen will be evaluated using changes in a composite efficacy outcome in order to assess the neurologic progression in participants. Highlight is that, it is the first study that uses quality of life rating. Moreover, assessment of biochemical markers of response and also due to a sufficient dosing duration will be performed to assess the effectiveness of HP-β-CD in NPC-1. This is a global, multi-site study with the largest planned number of participants, and this will allow a better knowledge about NPD-C and efficacy treatment.

- A Phase I/II study to evaluate the safety and pharmacokinetics of intravenous Trappsol Cyclo (HP-β-CD) in patients with Niemann-Pick disease type C (NPC-1) and the pharmacodynamic effects of treatment upon markers of cholesterol metabolism and clinical outcomes (see ClinicalTrials.gov Identifier: NCT02912793 and EudraCT Number: 2015-005761-23).

Phase 1/2, double-blind, randomized, multicentre, parallel group study based on data available and information from the administration via compassionate/named patient use in patients with NPC-1, and information of other cyclodextrin products in the literature. The study has two stages: the primary objective of Stage 1 is to compare the plasma pharmacokinetics (PK) of three different doses of IV HP-β-CD in the prevention/delay of NPC-1 progression whereas Stage 2 is to evaluate their efficacy and tolerability in the management of clinical

manifestations. Secondary objectives include investigation of the effect of three different doses of HP-β-CD IV upon serum and lymphocytic markers of cholesterol metabolism (Stages 1 and 2) and evaluation of concentrations in the CSF following IV administration (Stage 1), evaluation of the impact of treatment upon behavioral aspects and the impact of treatment upon measures of neurological function including ataxia, aphasia, and saccadic eye movements of NPC-1 (Stage 2). The outcome measures are: plasma and CSF concentrations of HP-β-CD following IV administration, serum cholesterol markers, global impression of disease, quality of life scores, change in NNCSS, and changes in hepatic and splenic morphology.

In order to achieve these objectives, the primary endpoint of Stage 1 is plasma concentrations (at 0, 2, 4, 6, and 8 hours after the start of infusion and 30 minutes, 1, 2, 4, 8, and 12 hours after the end of the infusion) of HP-β-CD during and following infusion to evaluate time to maximum concentration (T max), maximum concentration (Cmax), volume of distribution and elimination half-life ($t_{1/2}$). The primary endpoint of Stage 2 is the change from baseline in global impression of disease severity at 48 weeks and the proportion of patients at 48 weeks with a reduction from baseline of at least one point in two or more domains of the NNCSS.

Patients taking miglustat are not excluded of the study because this drug is an approved treatment for NPC-1 in Europe and it would be unethical, but it is planned to balance randomization across groups for its use.

This clinical trial is already recruiting patients in United Kingdom and it is planned to recruit 12 patients (3 children of 2–11 years, 3 of 12–17, and 6 adults 18–64 years). Patients will be randomized 1:1:1 to one of the three dose levels (1500, 2000, or 2500 mg/kg; four patients per dose level). Treatment will be administered over 8 hours by slow IV infusion at a concentration of 250 mg/mL every 2 weeks. Patients completing Stage 1 of the study will continue into Stage 2 and receive treatment for 48 weeks.

The design enables early assessment of biochemical markers of response and also due to a sufficient dosing duration, to assess the effectiveness of HP-β-CD in NPC-1 and its pharmacokinetics.

- A Phase I study to evaluate the single and multiple-dose pharmacokinetics of intravenous Trappsol Cyclo (HP-Beta-CD) in patients with Niemann-Pick disease type C (NPC-1) and the effects of dosing upon biomarkers of NPC disease. (See ClinicalTrials.gov Identifier: NCT02939547).

Phase I, double-blind, randomized, single-center, parallel group study based on information and data available from the administration of HP-β-CD via compassionate/named patient use in patients with NPC-1, and data on other cyclodextrin products in the scientific literature.

The study has a first phase of screening (up to 4 weeks), a treatment phase of 12 weeks and a later phase of follow-up of 4 weeks. The primary objective is to compare the plasma pharmacokinetics of single and multiple doses of two different levels of IV HP-β-CD. Secondary objectives include investigation of the effect of different doses of IV HP-β-CD upon serum and

lymphocytic markers of cholesterol metabolism and evaluation of HP-β-CD concentrations in the CSF following IV administration, evaluation of the impact of treatment upon measures of neurological function including aphasia, ataxia, and saccadic eye movements, and the impact of treatment upon behavioral aspects of NPC-1.

This study is currently recruiting participants. It is planned to recruit a total of 12 patients (all adults) which will be randomized 1:1 to one of the two dose levels (1500 mg/kg or 2500 mg/kg; 6 patients per dose level). Treatment will be administered every 2 weeks by slow IV infusion over 8 hours. Patients will receive treatment for a total of 12 weeks.

As primary outcome measures are pharmacokinetics parameters: Tmax, Cmax, volume of distribution, and $t_{1/2}$ of HP-β-CD in plasma from NPC-1 patients by measurement at pre-infusion then 2, 4, 6, 8, 8.5, 9, 10, 11, 12, 16, and 20 hours after the start of the infusions at weeks 1 and 12.

The design of the proposed study thus enables a better knowledge about pharmacokinetics of IV HP-β-CD administration, an early assessment of potential biochemical markers of response but allows for a sufficient dosing duration to enable the short-term effectiveness of HP-β-CD in NPD-C to be assessed.

In conclusion, there is a published clinical trial results, using IT administration of HP-β-CD and shows moderate response thought slowed disease progression with an acceptable safety profile. Another IT HP-β-CD clinical trial and two IV HP-β-CD clinical trials are ongoing but not results are been published yet. Regarding the route of administration, exist a debate, and treatment with HP-β-CD has used four different paradigms: IV only, IV followed by the addition of IT sequential, IV and IT initiated concurrently, and IT only. The main reason for IT route is that HP-β-CD does not cross the BBB, however, in animal models systemic HP-β-CD positively affects CNS disease thus CNS penetration may not be essential for neurologic efficacy. The ongoing clinical trials will lead to an improvement in knowledge of HP-β-CD for NPD-C treatment, setting the best route, dose and posology for NPD-C patients.

7. Experience in humans: potential toxicities of the different administration routes

There are three possible administration routes for HP-β-CD: IV, IT, or ICV via an Ommaya reservoir. Some adverse events observed could be related to the administration route, the disease progression, or the cyclodextrin itself.

The review performed by the EMA regarding the use of HP-β-CD as an excipient indicated that the IV administrations had low toxicity, being the most prevalent issue of the renal toxicity, especially with high doses [51]. However, any patients showed renal toxicity with HP-β-CD therapy [32]. This was the first route employed, but later a change to IT route was requested due to the discovery that HP-β-CD not cross the BBB and only a little quantity is able to enter to the brain [21, 24, 25, 30].

Based on animal models, ICV route was proposed as an alternative with potential benefits and this route was chosen in the Phase I clinical trial (NCT01747135) using an Ommaya reservoir to facilitate the administration. Unfortunately, complications due to colonization by *P. acnes* led to change ICV route to IT [52]. Currently, IV route is being tested in two ongoing clinical trials, whereas IT route is analyzed in another ongoing trial and in the recently published trial [50].

Megías-Vericat et al. reviewed the initial published cases of HP-β-CD treatment [32]. Regarding safety, 11 of 17 NPD-C patients included suffered AEs. Of the 17 AEs reported, 6 were related to the route of administration, specifically with the IT and ICV routes and 10 of them could be attributed by HP-β-CD. Six AEs associated to the route were: CNS bleed related to insertion of the Ommaya reservoir system in a patient treated with ICV HP-β-CD led to ICV administration suspended [34]; post lumbar puncture headache and vomiting resolved with switching a Whitacre spinal needles [41–43]; post lumbar puncture pain, headache, nausea, and vomiting resolved with symptomatic and postural treatment [44]; seizures with fever resolved without changes in treatment [40] and aspiration pneumonia; febrile syndrome; and candidiasis resolved with antibiotic and antifungal treatment [49].

Among AEs related to HP-β-CD itself, loss of hearing was reported in four reported cases of IT infusions [41–45], although it proved reversible in two of them. These patients were administered with reduced doses of HP-β-CD after hearing recovery, two times in one of them. In other two patients, despite delaying the next dose, hearing loss was not reversed. This AE was also observed in animal studies with IT [18], ICV [53], and intracisternal [25] routes of administration. At the dosage employed, hearing loss is an expected AE, as well as a well-known NPD-C symptom. At the clinical trial recently published, loss of hearing was reported in all the participants [50].

Four patients suffered from fever two or more times after HP-β-CD administrations [37, 40, 46, 49]. In one case, fever was accompanied by seizures [40], whereas another patient showed an infusion reaction with fever and transient diffuse pulmonary cloudiness [37]. In one patient, after intermittent fever episodes, a diagnosis of chemical meningitis was made after bacterial meningitis was discarded. The IT-OR was withdrawn, and HP-β-CD was reintroduced 2 months later because the patient's condition worsened [46]. Other patient showed two episodes of chemical meningitis (bacterial meningitis was discarded in both cases) after IT and IT-OR HP-β-CD administrations, although both were resolved quickly and without consequences [49]. This AE could be related to the method of administration or disease symptoms and not to HP-β-CD. However, the chemical meningitis observed seems to be associated to the drug after analyzed with the Naranjo algorithm [49]. Some acute neurological effects after the infusion of high doses that resolve after few days were described by some investigators in conferences (but unpublished yet).

Regarding the results of the first clinical trial published, no serious AEs were observed [50]. Marked expected AE included: ototoxicity (14 of 14 patients) and post lumbar puncture headache (9 of 14 patients). Among unexpected AE included, post-administration unsteadiness and fatigue occurs at doses above 600 mg. The degree of impairment varied between subjects but usually was transient and occurred 24–72 hours after administration.

Sensorineural hearing loss was present in all subjects of NIH-cohort (14 patients) and 2 of 3 RUMC-cohort, and according to the study results, it was associated with the administration of HP-β-CD. Moreover, the data obtained suggest that there is greater HP-β-CD ototoxicity in subjects who have not yet lost hearing due to NPD-C itself. Also, tinnitus was present in 6 of 14 patients in NIH-cohort and 1 of 3 in RUMC-cohort [50].

8. Conclusions

Until now, NPD-C treatment has been supportive and symptomatic with miglustat as the only disease-specific drug approved in some countries. HP-β-CD is a new option under investigation with two different products, Kleptose® and Trappsol Cyclo®, which are not chemically equivalent and therefore may not be biochemically equivalent or lead to comparable formulations from a clinical development perspective.

Based on preclinical animal studies, HP-β-CD has been tested in humans affected by NPD-C during the last years. In compassionate use outside clinical trials, HP-β-CD has been administrated with either IV, IT, or ICV. Despite the positive results observed with IV HP-β-CD, there is no clear evidence to date that IV route has a clinical benefit in CNS symptoms of NPD-C as most of the reports combined IV and IT/ICV administrations. The reported cases of IT infusions obtained higher improvements reducing the disease progression.

The results of a recently published clinical trial reproduced the findings observed with IT route. The trial has shown slowing of NPD-C progression in 14 patients with a dose escalation of IT HP-β-CD administrated monthly as well as in 3 patients with administration every 2 weeks regarding a historical comparison cohort.

Some adverse events observed could be related to the administration route, the disease progression, or the cyclodextrin itself. The safety profile of HP-β-CD seems acceptable, being the loss of hearing (related to HP-β-CD) the most frequent adverse reported in the clinical trial and published cases. However, some severe toxicities have been reported including chemical meningitis and fever although not in published clinical trial.

Furthermore, there are currently two IV HP-β-CD and one IT HP-β-CD ongoing clinical trials without published results. The findings of these trials will lead to an improvement in knowledge of HP-β-CD for NPD-C, setting the best route, dose, and posology. Currently, the short experience with HP-β-CD suggested that it could be effective in the management of NPD-C but the results of ongoing clinical trials will be definitive.

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