Effects of intracerebroventricular administration of 2-hydroxypropyl-β-cyclodextrin in a patient with Niemann–Pick Type C disease

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ARTICLE INFO

ABSTRACT

Niemann–Pick Type C disease (NPC) is an autosomal recessive lysosomal storage disorder characterized by progressive neurological deterioration. Previously, we reported that intravenous administration of 2-hydroxypropyl-β-cyclodextrin (HPB-CD) in two patients with NPC had only partial and transient beneficial effects on neurological function. The most likely reason for HPB-CD not significantly improving the neurological deficits of NPC is its inability to cross the blood–brain barrier. Herein, we describe the effects of intrathecal HPB-CD in an eight-year-old patient with a perinatal onset of NPC, administered initially at a dose of 10 mg/kg every other week and increased up to 10 mg/kg twice a week. Clinically, the patient maintained residual neurological functions for two years, at which time nuclear magnetic resonance spectroscopy showed a decreased choline to creatine ratio and increased N-acetylaspartate to creatine ratio, and positron emission tomography revealed increased standardized uptake values. Total-tau in the cerebrospinal fluid (CSF) was also decreased after two years. No adverse effects were observed over the course of treatment. The CSF concentrations of HPB-CD during the distribution phase after the injections were comparable with those at which HPB-CD could normalize

cellular cholesterol abnormality in vitro. Further studies are necessary to elucidate the mechanisms of action of HPB-CD in NPC, and to determine the optimal dose and intervals of HPB-CD injection.

1. Introduction

Niemann–Pick Type C disease (NPC) is an autosomal recessive lysosomal storage disorder characterized by progressive neurological deterioration. NPC is caused by mutations in either the *NPC1* or *NPC2* gene, both of which encode proteins involved in regulating intracellular lipid trafficking. These gene mutations lead to marked lysosomal accumulation of unesterified cholesterol and several glycosphingolipids [1].

Recent studies in NPC mice showed that hydroxypropyl-β-cyclodextrin (HPB-CD) injections are effective in treating the disease [2–5]. Cyclodextrins (CDs) are cyclic oligosaccharides known as host molecules that form inclusion complexes with guest molecules, including exogenous and endogenous lipophiles, and are widely used in food, cosmetics, and pharmaceuticals [6]. HPB-CD has high aqueous solubility and extremely low toxicity, and is already used clinically with other drugs in parenteral preparations. Previously, we reported the effectiveness of HPB-CD administered intravenously in two patients with NPC [7]. It had only partial and transient beneficial effects on neurological function, probably due to issues with crossing the blood–brain barrier [4]. Recently, direct administration of HPB-CD into the cerebral ventricle of NPC model mice normalized the biochemical abnormalities and completely prevented the expected neurodegeneration, and Phase I Clinical Trial of intracerebroventricular (ICV) HPB-CD administration began in February 2013 [4,8]. Thus, we tested the effectiveness and safety of intrathecal administration of HPB-CD in a patient with NPC.

2. Patient and methods

2.1. Patient

A girl in whom hepatosplenomegaly was detected before birth, was diagnosed with NPC based on mutations in the *NPC1* gene (c.581_592delinsG, Y1088C) at the age of two months. She developed slowly until 3 years of age, walking alone at 19 months and using two-word sentences at 3 years. However, after 3 years of age she started to exhibit rapid neurological deterioration, including progressive ataxia, cataplexy, dysarthria, dysphagia, and convulsions. She was started on intravenous HPB-CD treatment at 4 years of age, by which stage she had marked hepatosplenomegaly, could walk indoors with assistance, and speak only a few unclear words. She also exhibited vertical gaze palsy, mild occasional dysphagia, slight hypotonia, ataxia, frequent attacks of cataplexy, and rare convulsions. After one year of treatment with HPB-CD, the hepatosplenomegaly was slightly improved, but unfortunately, her neurological signs had worsened as she gradually developed dysphagia, rigidity, and frequent seizures. Consequently, she became bed-ridden and lost the ability to speak. She was started on tube feeding via a gastrostomy. We added miglustat after one year of treatment with HPB-CD; however, her neurological deterioration continued. Her swallowing function worsened, and she suffered from severe aspiration pneumonia. Her head MRI also showed progressive brain atrophy (Fig. 2).

2.2. Intrathecal administration of HPB-CD

After two years of intravenous HPB-CD treatment, intrathecal HPB-CD therapy was started at the age of 6 years. With informed consent from the parents, we initiated a 10 mg/kg dose of 20% HPB-CD diluted with 6 mL saline administered via lumber puncture every other week. Two months later, no adverse effects were observed, thus we implanted an Ommaya reservoir to administer 20 mg/kg HPB-CD weekly thereafter (Table 1). The dose was increased by up to 450 mg (22.5 mg/kg) every week. Fifteen months later, we changed the treatment schedule to a 200-mg dose twice a week according to the measured concentrations of HPB-CD in the cerebrospinal fluid (CSF) (Table. 2). Twenty-one months later, we transiently increased the dose of HPB-CD up to 300 mg twice a week, but reduced it again shortly after due to an increased total tau in the CSF. Intravenous administration of HPB-CD was stopped 12 months after the start of intrathecal HPB-CD

Table 1
The dose and intervals of HPB-CD.

	IV HPB-CD	ICV HPB-CD	Interval	Weight
0–2 M	20 g × 2/week	165 mg	Every 2 weeks	16.5 kg
3 M–4 M	20 g × 2/week	330 mg	Every 2 weeks	15.7 kg
5 M–6 M	20 g × 2/week	450 mg	Every 2 weeks	18.5 kg
7 M–14 M	20 g × 1/week	450 mg	Every week	20.0 kg
15 M–20 M		200 mg	Twice a week	21.3 kg
21 M–22 M		300 mg	Twice a week	22.4 kg
23 M–		200 mg	Twice a week	24.0 kg

treatment, whereas miglustat was continued. The treatment protocol used in the present study was approved by the Ethics Committees of Saga University (no. 2009-05-04) and Kumamoto University (no. 608).

Clinical assessments were performed before the start of treatment and then after 3, 6, 12, 18, and 24 months of treatment, comprising blood and CSF testing, head magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), electroencephalography (EEG), auditory brainstem evoked potentials (ABR), and videofluoroscopy. CSF levels of total tau (T-tau) were also measured by the ELISA Kit (R&D, Minneapolis) according to the manufacture's protocol to determine axonal damage.

2.3. Determination of HPB-CD concentrations in CSF

CSF concentrations of HPB-CD were determined by high-performance liquid chromatography according to the method of Frijlink et al. [9] with slight modifications. HPB-CD was purchased from Roquette Japan K.K. (Osaka). The average degree of substitution of 2-hydroxypropyl groups was 4.41, and the average molecular weight was 1391 Da. All other chemicals used were of analytical grade.

The HPLC system consisted of two LC-10A pumps (Shimadzu, Tokyo), a SIL-10A XL auto injector (Shimadzu), and a SPD-10A UV–VIS absorbance detector (Shimadzu). Capillary tubing of 10 m (1.0 mm i.d.) was used for mixing the column eluent with the post-column reagent.

We added 100 μL of CSF sample to 40 μL of 20% trichloroacetic acid. After mixing and centrifugation at 10,000 *g* at 4 °C for 5 min, 40 μL of 1 M sodium carbonate solution was added to 80 μL of the clear supernatant. The sample was passed through a filter (MILLEX-HP PES 0.45 μm), and 10 μL of the filtrate was injected onto the HPLC column. The analytical column was a OHpak Shodex column (SHOWA DENKO K.K., Tokyo, 300 × 8.0 mm i.d.), used with a guard column. The column eluent was a sodium chloride solution (0.9%) adjusted to pH 4.3 by acetic acid, with a flow rate of 0.9 mL/min. The post-column reagent was 8 mM sodium carbonate and 60 μM phenolphthalein in water, and the flow rate was 0.9 mL/min. The effluent was monitored at 546 nm. The detection limit of HPB-CD in CSF was 20 μg/mL.

3. Results

3.1. Clinical symptoms and signs

Clinical symptoms and signs remained virtually stable during two years of intracerebroventricular HPB-CD treatment. Although the patient's facial expression is slightly impaired two years later, she can still laugh with

Table 2
Concentrations of HPB-CD in the CSF after the intrathecal injection of HPB-CD at a dose of 200 mg or 300 mg to the patient with NPC.

Age of the patient (year)	Body weight of the patient (kg)	Dose of HPB-CD (mg)	Concentration of HPB-CD in the CSF[a] (μg/mL)		Apparent volume of distribution[b] (mL/kg)
			15 min after injection	1 h after injection	
7.58	20.1	200	1106 ± 24	205 ± 23	5.1
7.75	23.1	300	2122 ± 28	284 ± 25	3.1

[a] The mean ± S.E.M. of three determinations of the same sample.
[b] Estimated by the dose of HPB-CD and the CSF concentration extrapolated immediately after the injection.

voice and horizontal eye movements have been preserved. Swallowing function assessed by videofluoroscopy was slightly improved at 18 months after treatment started, but became slightly worse at 24 months (Supplemental data 1). At 21 months from the beginning of intrathecal HPB-CD, she received the SynchroMed-2 pump implant to start intrathecal baclofen (ITB) therapy for increased rigospasticity of her trunk and limbs. She suffered from aspiration pneumonia immediately after the implantation operation. Seizure frequency was not changed significantly.

Frontal dominant, irregular, high-voltage, slow activities on the awake EEG were significantly decreased after two years of treatment with intrathecal HPB-CD (Fig. 1), although there were no significant changes on brain MRI (Fig. 2). MRS of the cerebral white matter, basal ganglia, and cerebellum showed a decreased choline to creatine ratio (Cho/Cr), indicating decreased myelin destruction, and an increase in the N-acetylaspartate to creatine ratio (NAA/Cr), indicating decreased neuronal and axonal damage in all areas (Fig. 3A,B). The standardized uptake values on PET were slightly increased in all areas after two years (Fig. 4A, B), indicating increased metabolism and activities of the patient's brain. There were no changes in ABR.

3.2. Changes in CSF T-tau

Changes in CSF T-tau are represented in Fig. 5. The levels of CSF T-tau gradually decreased after the beginning of intrathecal HPB-CD therapy, but were transiently increased from the 21 months to 23 months period, when the dose of HPB-CD reached 300 mg twice a week.

3.3. HPB-CD concentrations in CSF

Table 1 details the concentrations of HPB-CD in CSF 15 min and 1 h after the intrathecal injection of HPB-CD at a dose of 200 mg (10.0 mg/kg) or 300 mg (13.0 mg/kg). At these doses, the HPB-CD concentration ranged from 2122 μg/mL (1.53 mM) to 205 μg/mL (0.15 mM) during 1 h. The apparent volumes of distribution for HPB-CD were estimated to be 5.1 mL/kg at a dose of 200 mg and 3.1 mL/kg at a dose of 300 mg, respectively.

4. Discussion

Intravenous HPB-CD treatment has only partial and transient beneficial effects on neurological function, whereas direct administration of HPB-CD into the cerebral ventricle of NPC model mice normalizes the biochemical abnormalities and completely prevents the expected neurodegeneration [4]. Herein, we also demonstrated the efficacy of ICV administration of HPB-CD in a patient with NPC. Unfortunately, it was difficult to improve the clinical symptoms because our patient was already progressed at the beginning of ICV HPB-CD therapy. However, she could maintain residual functions such as horizontal gaze pursuit and laughing with voice, and swallowing function at 18 months was improved. Taking into account the rapid progression of neurological dysfunction in our patient after two years of treatment with intravenous HPB-CD and miglustat, the stable clinical state for two years with ICV HPB-CD indicates the effectiveness of such therapy. Furthermore, EEG, MRS and PET study showed beneficial effects.

Increased levels of T-tau in CSF have been reported in NPC patient [10,11], indicating axonal degeneration. The CSF T-tau levels of our patient were decreased, except for one transient increase at 20 to 22 months after the beginning of ICV HPB-CD therapy. Interestingly, the reduced levels of NAA/Cr in MRS, which indicates degeneration of neurons and axons, changed reciprocally with the CSF T-tau levels. Although increased NAA/Cr and reduced T-tau have been reported in miglustat therapy, ICV HPB-CD was probably the major effective contributor to improvements in this case because miglustat had been started one year before the beginning of the ICV HPB-CD and the patient's neurological conditions deteriorated during the pre-ICV HPB-CD period.

The major concern with direct administration of HPB-CD into CSF is adverse effects, especially in auditory function, and dose-dependent auditory toxicity from HPB-CD has been reported in cats [12]. Specifically, weekly subcutaneous injections of 4 g/kg HPB-CD and every other weekly intrathecal injections of 120 mg HPB-CD in NPC cats increased the hearing threshold. Fortunately, our patient showed no adverse
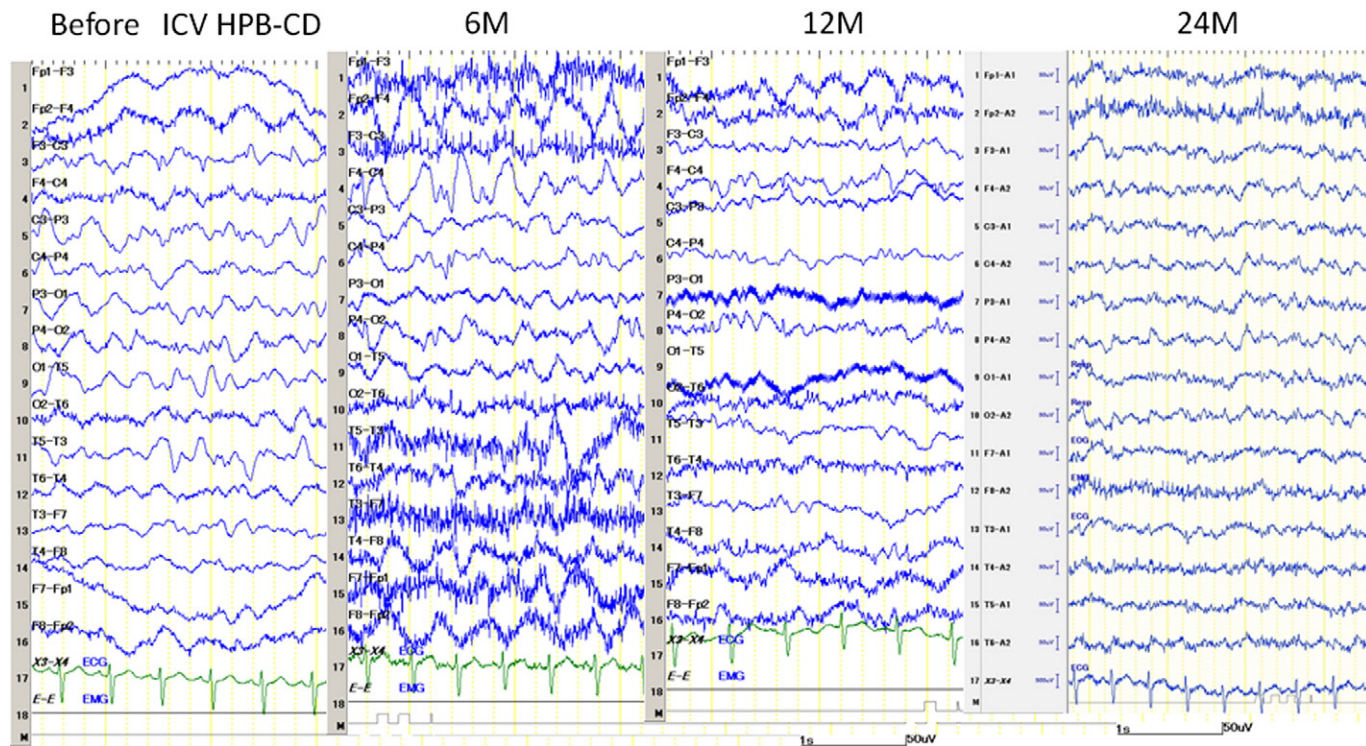
Fig. 1. Changes in the awake EEG before and after treatment with intrathecal 2-hydroxypropyl-β-cyclodextrin. Frontal-predominant, irregular, high-voltage, slow activities were significantly decreased after two years of treatment.
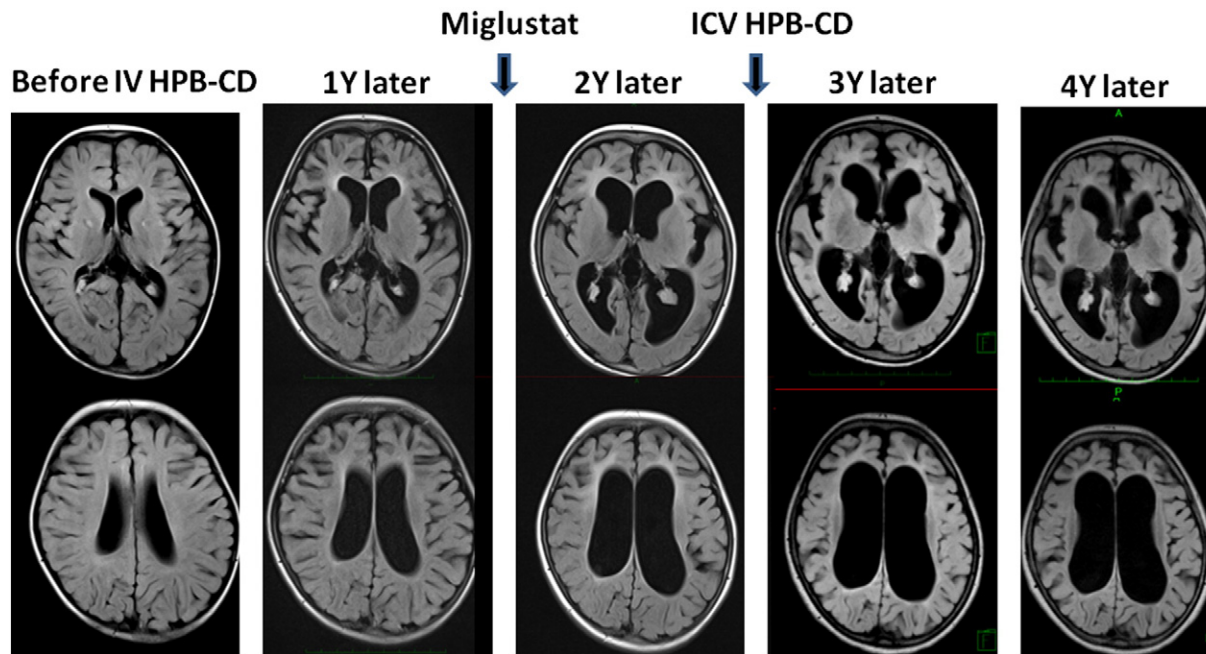
Fig. 2. Changes in MRI (FLAIR, fluid attenuated inversion recovery, images) before and after treatment with 2-hydroxypropyl-β-cyclodextrin. Miglustat was added after 1 year of treatment. Progressive brain atrophy is visible especially before the ICV HPB-CD therapy.
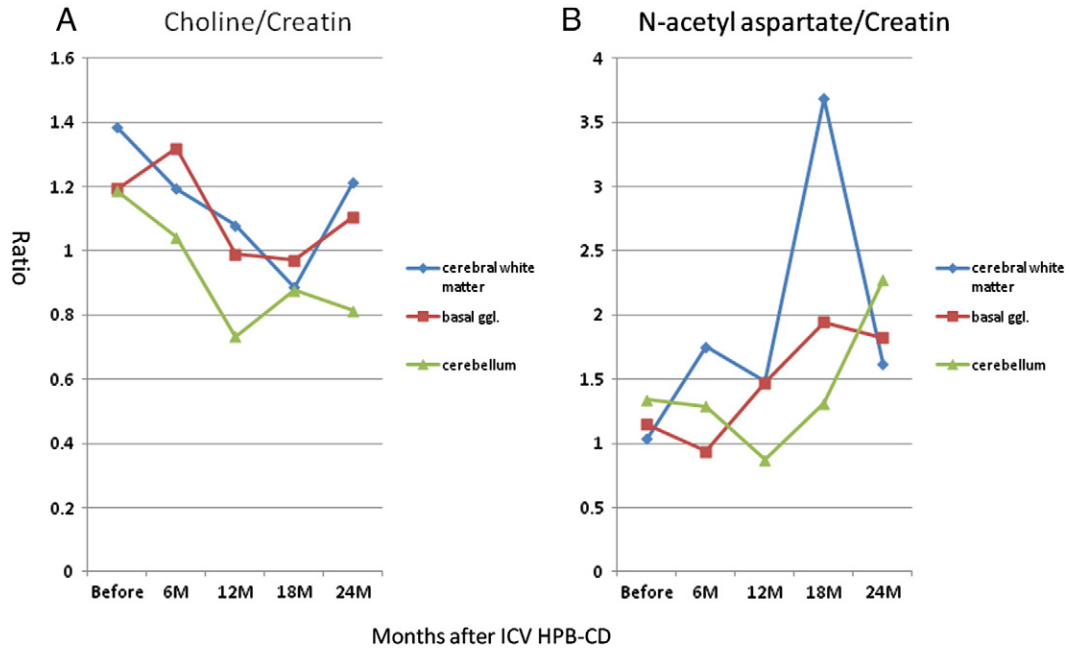
Fig. 3. (A) Changes in the choline to creatine ratio (Cho/Cr) of MRS in the cerebral white matter, basal ganglia, and cerebellum. (B) Changes in the N-acetylaspartate to creatine ratio (NAA/Cr) of MRS in the cerebral white matter, basal ganglia, and cerebellum.
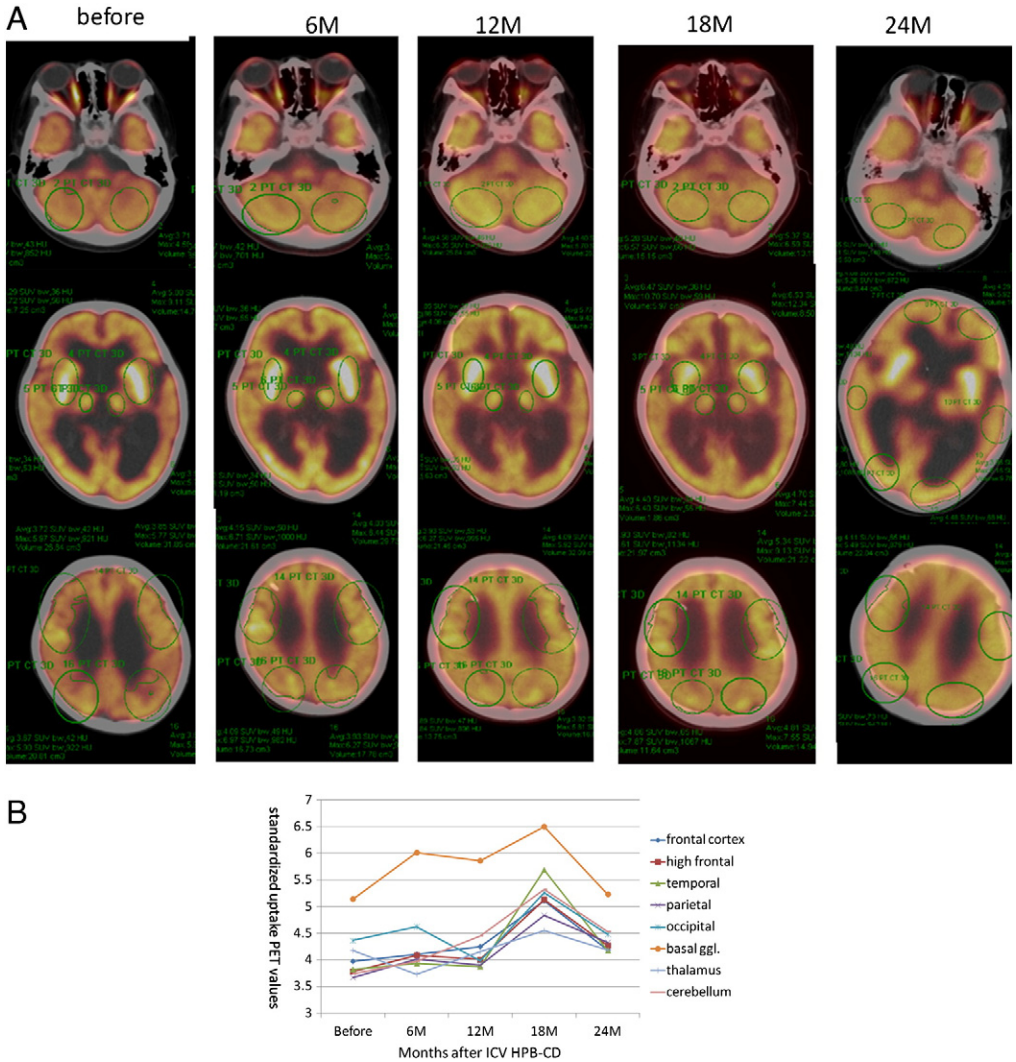
Fig. 4. (A) Changes in PET images after ICV HPB-CD. The cerebral cortex and cerebellum looks brighter than before. (B) Changes in standardized uptake PET values after ICV HPB-CD. The uptake values reached the peak 18 months after ICV HPB-CD in every region.

effects from ABR studies even after two years of treatment with frequent intrathecal administration (Data not shown).

A recent study using sensitive liquid chromatography–tandem mass spectrometry described the concentrations of HPB-CD in CSF and plasma after an intrathecal injection of HPB-CD at a dose of 50 mg in 3 patients with NPC, where the clearance, volume of distribution, and half life were 18–19 mL/h, ~130 mL, and 6.58–10.1 h, respectively [13]. Due to the limited sensitivity of our analytical methods in the present study, the concentrations of HPB-CD in CSF seemed comparable to those with initial distribution phase after the intrathecal injection. During the distribution phase after the ICV injection, the concentrations of HPB-CD in CSF were in the range at which HPB-CD could normalize cellular cholesterol abnormality in vitro [14,15]. In addition, the apparent volumes of distribution of HPB-CD estimated from the distribution phase were slightly larger than the CSF volume in children [16]; the volume of distribution of HPB-CD at steady state should be larger than the apparent volume. This indicates that following the ICV injection,
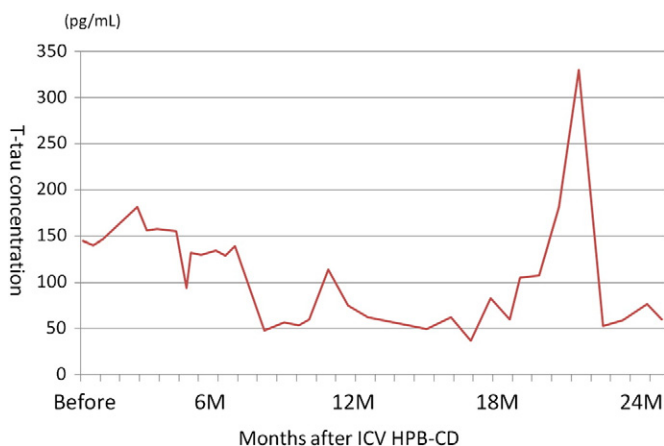
Fig. 5. Changes in CSF T-tau concentration after ICV HPB-CD therapy. The levels of CSF T-tau gradually decreased after the ICV HPB-CD therapy, but were transiently increased from the 21 months to 23 months period, when the dose of HPB-CD reached 300 mg twice a week.

HPB-CD penetrates deeper into the central nervous system beyond the CSF compartment, as suggested by Ottinger et al. in an animal study [8].

The reported ED_{50} in NPC mice is approximately about 0.5 mg/kg, with 35 mg/kg administration of HPB-CD effective for 1 week [4]. An in vitro study using primary cultures of neurons and glial cells from $Npc1^{-/-}$ mice also demonstrated that 0.1 mM of HPB-CD is the optimum concentration and that 10 mM is toxic for cells [15]. Our present data combined with these previous observations suggest that 10 mg/kg would be a sufficient dose of ICV HPB-CD, although the optimum intervals for ICV injections of HPB-CD remain unclear. In our experience, the patient looks more alert after several days of HPB-CD injection according to the parents' observation. Thus, we tried twice a week injections of 200 mg HPB-CD. MRS and PET study suggested better results, supported by the observation of slightly lower clearance rates of HPB-CD compared to the CSF turn-over rate of 21–25 mL/h in normal human subjects [13].

In the present study, we showed the efficacy and safety of ICV HPB-CD treatment and the effect on concentrations of HPB-CD in CSF. However, the optimal dose and dosing intervals for ICV HPB-CD remain to be determined, necessitating further studies into the mechanisms of HPB-CD action in NPC.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgmr.2014.08.004.

Acknowledgments

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