Substrate Reduction Therapy in Four Patients with Milder *CLN1* Mutations and Juvenile-Onset Batten Disease Using Cysteamine Bitartrate

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Abstract Homozygous mutations in the gene *CLN1* typically result in infantile-onset neuronal ceroid lipofuscinosis, a severe progressive neurological disorder with early death. The gene *CLN1* encodes the enzyme palmitoyl protein thioesterase (PPT1), which is involved in lysosomal degradation of S-fatty acylated proteins. Cysteamine bitartrate (Cystagon) has been shown to reduce the storage material in PPT1 deficient cells. We report the results of a 7-year, open label, nonrandomized trial using Cystagon in four individuals with juvenile-onset NCL resulting from milder *CLN1* mutations. The Cystagon doses were gradually increased with the goal of achieving 50 mg/kg bodyweight. The disease progression was monitored with parental questionnaires in four treated individuals and five untreated controls with the same *CLN1* mutations. Mononuclear leukocytes from the treated individuals were examined for submicroscopic lysosomal storage inclusions. Cystagon treatment resulted in decreased storage material in peripheral leukocytes of the treated individuals. No severe side effects were noted. An allergic rash occurred in one of the individuals that required a dose reduction. The treatment did not result in overall attenuation of the disease progression. Slower progression of the disease was observed in two of the individuals when they were analyzed separately. However, slower progression in these individu-

als was also observed prior to starting the treatment. This effect may have been due to the higher Cystagon dose achieved in this group, but it could also have been coincidental. The apparent lack of toxicity of Cystagon may warrant further Cystagon trials in infantile NCL, possibly in conjunction with other developing therapies.

Introduction

The neuronal ceroid lipofuscinoses (NCL), also referred to as Batten disease, include at least 13 progressive genetic disorders, characterized with neurodegeneration and early death. A unifying feature of these devastating conditions is the lysosomal accumulation of autofluorescent lipopigment (Hobert and Dawson 2006). Homozygosity for inactivating mutations in the gene *CLN1* (encoding PPT1) typically results in the severe infantile form of NCL with an onset within the first 2 years of life, a fast downhill course, and early death (Mole et al. 2010). Milder *CLN1* mutations may present with later onset NCL at various ages (Das et al. 1998). PPT1 is a lysosomal enzyme that is abundant in the brain, and removes long chain fatty acids bound to cysteine residues of S-fatty acylated proteins (Hofmann et al. 1997). Cysteamine bitartrate (Cystagon), a lysosomotropic agent, has been shown in vitro to inhibit the formation of cysteine thioesters, the storage material in PPT1 deficient cells (Zhang et al. 2001). It was thus suggested that Cystagon might be used as a substrate reduction agent in infantile NCL due to *CLN1* mutations (Zhang et al. 2001). This effect on substrate accumulation was not replicated in later experiments (Lu and Hofmann 2006). Cystagon has been successfully used since the 1980s in the treatment of another progressive lysosomal storage condition, cystinosis.

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Table 1 Treated and control individuals included in the Cystagon protocol

Patient ID	CLN1 mutation	Age of first manifestations	Ages of treatment	Dose range
1-2	cA223C homozygote	8 years	16–24 years	30–50 mg/kg
1-6	gT125C homozygote	3 years	8–11 years	4.7–25 mg/kg
1-9	cC451T/cG749T compound heterozygote	5 years	13–18 years	17–38 mg/kg
1-10	cA223C homozygote	8 years	13–18 years	30–50 mg/kg
1-5	gT125C homozygote	6 years	Control	Control
1-7	cC451T/cG749T compound heterozygote	6 years	Control	Control
1-8	cC451T/cG749T compound heterozygote	6 years	Control	Control
1-11	cA223C homozygote	7 years	Control	Control
1-12	cA223C homozygote	6 years	Control	Control

The beneficial effect of Cystagon in the treatment of cystinosis is due to its ability to decrease/clear the abnormal lysosomal accumulation of cystine associated with this disorder (Goodyer 2011).

The lysosomal storage disorders, including NCL, are progressive conditions. Specific disease-modifying treatments in these conditions has been typically associated with better therapeutic outcomes when such treatment was initiated earlier in the course of the disease, and best prior to the onset of clinical manifestations. *CLN1* mutations are typically associated with infantile-onset NCL and a very fast progression that leads to early death between the ages of 2 and 9 years (Mole et al. 2010). Late-onset (atypical) forms of *CLN1* disease were previously described. These milder *CLN1* variants are associated with later disease onset and milder clinical course that more closely resembles juvenile NCL due to *CLN3* mutations (Das and Becerra 1998). Patients with such atypical forms of *CLN1* disease are better candidates for a pilot trial of specific disease treatment since they are often diagnosed at an earlier stage of the disorder, when stabilization/slowing of disease progression may be easier to achieve.

Patients and Methods

Patients Included in the Study

Four individuals, homozygous for atypical *CLN1* mutations and with absent or very low PPT1 activity were included in this study. The treated individuals' mutations, age at initial presentation, and age of inclusion in the study are summarized in Table 1. The clinical manifestations in all individuals studied started after the age of 3 years and on initial evaluations were suggestive of the juvenile type of NCL. The two treated individuals, who had siblings previously diagnosed with NCL, had clinical manifestations of the disease reported at an earlier age than their siblings. This may be due to an increased parental awareness of the

early signs and symptoms of the disease. Some of the data on one of the treated individuals were previously reported (Wen et al. 2012). The study was conducted under an IRB-approved protocol, and all participants were included after an informed consent was signed.

Nontreated Controls

Five control individuals, who had the same *CLN1* mutations as the treated individuals, were included in this study. Three of these individuals were older siblings of the treated individuals. Two were unrelated to the treated individuals. Mutations and age of onset of the control individuals are shown in Table 1.

Groups of Monitored Individuals

The treated subjects and the controls were divided into three observational groups consisting of individuals with identical mutations within each group. Three groups were established as follows:

- Group 1 included two treated and two control (untreated) individuals, who are homozygous for *CLN1* mutation c.223A>C.
- Group 2 included one treated individual and one untreated sibling, who are homozygous for *CLN1* intronic mutation g.125T>C.
- Group 3 included one treated and two untreated siblings, who are compound heterozygotes for the *CLN1* mutations c.749G>T/451C>T.

Treatment Regimen

Criteria for inclusion in the study were the ability to swallow and eat without the assistance of a gastric tube, and the lack of known allergies to Cystagon or penicillamine.

The initial evaluation consisted of general physical, neurological, and genetic evaluations.

The initial evaluation included CBC, SMA18, and electron microscopy study of peripheral white blood cells for inclusions. An EEG and brain MRI were also performed. After the initiation of the Cystagon treatment, the patients were evaluated at 3, 6, and 12 months during the first year, and then every 6 months. All evaluations, blood tests, brain MRIs, and EEGs were repeated every 6 months. The initial doses of Cystagon were 25–30 mg/kg that were gradually increased with a goal of 40–50mg/kg. In patients 1-6 and 1-9 (Table 1), this could not be achieved because of the discomfort associated with the unpleasant taste of Cystagon and/or because of the side effects of the medication (see [Results](#)). The age of the treated patients when the treatment was conducted is shown in Table 1.

Disease Severity Scores (DSS)

In order to monitor the disease progression and severity, disease severity scores (DSS) were established for every year of life of each treated and control individual. The previously established disease severity scale by Kohlschutter et al. (1988) was used with one modification: one additional scored parameter – psychiatric manifestations (behavior) was added. The additional parameter was also scored in scale 0–3 as were the other parameters. This scale is in use in the Batten Disease Registry in our Institute. The scoring for every year of life was done by the patients' parents. Simple, nonmedical language instructions for scoring were developed (see parental questionnaire in [Supplement 1](#)). Total DSS were obtained for every treated or control individual and for every year of life by adding the scores for all parameters.

Evaluation of Lysosomal Inclusions with Transmission Electron Microscopic (EM) Examination

Three of the treated individuals (patients 1-2, 1-6, and 1-9) had peripheral blood mononuclear leukocytes studied with electron microscopy (EM) for NCL-associated cytoplasmic inclusions at about 6 months intervals as previously described (Anderson et al. 2006).

Between 200 and 250 peripheral blood cells were studied on every specimen.

Results

Adverse Reactions

Minimal adverse reactions after the start of the Cystagon treatment were observed. One of the study participants (individual 1-9) had an allergic rash after starting the treatment. This resolved after the Cystagon dose was reduced. In addition, bad breath and lightening of the hair

color were of concern during the Cystagon use in all participants. It is important to point out that maintaining the targeted dose of Cystagon required swallowing of large numbers of capsules every day. The parents were instructed to mix the content of the capsules with food. This may have created some degree of discomfort in the participants related to the unpleasant taste of Cystagon. An electroencephalogram (EEG) was performed prior to the initiation of Cystagon and every 6 months while on treatment. All EEGs revealed nonspecific abnormalities during wakefulness – an excess of diffuse irregular slow waves, rhythmic bilateral slow waves, or slowing of the posterior background. In individual 1-2, the posterior background was gradually lost as the disease progressed and epileptiform discharges (spikes and sharp waves) appeared later. In Individual 1-6, the waking background activity slowed over time and epileptiform discharges also emerged. In individual 1-9, frequent generalized and multifocal epileptiform discharges were seen at the outset both spontaneous and induced by photo stimulation and electrographic seizures were seen on two occasions. Patients 1-2, 1-6, and 1-9 all developed clinical seizures during the trial and were placed on anti-seizure medications.

An MRI was performed prior to the initiation of Cystagon and every 6 months while on treatment. Cerebellar atrophy was shown on all MRIs. Routine blood work including sodium, potassium, chloride, glucose levels, and hemoglobin, hematocrit, WBCs, RBCs, and platelets were performed prior to the initiation of Cystagon and every 6 months while on treatment and were all within normal limits with no significant changes from baseline.

Lysosomal Inclusions

The results of the EM studies and the Cystagon doses are shown on Fig. 1. The proportion of cells with inclusions and the Cystagon doses were plotted against the participants' age. A tendency towards decrease of the proportion of cells with storage inclusions correlating with the dose of Cystagon is apparent in all three participants that were monitored. A temporary increase of the storage inclusions in the peripheral cells of individual 1-2 was observed after a period when Cystagon administration was temporarily discontinued for about 6 months. Overall, these studies demonstrate that Cystagon use was associated with decrease in storage material in the peripheral blood cells of the treated individuals.

Questionnaire Results

Results of the Disease Severity Scoring of the treated patients and nontreated controls are shown on Fig. 2. The disease severity scores (DSS) were obtained from the completed disease questionnaire. The score of all six

Fig. 1 Proportions of peripheral blood cells with NCL-specific inclusions identified on electron microscopy (EM) studies in three of the treated study participants are shown in relation to the Cystagon doses.

Such EM studies were not done for participant 1-10. The ID number of each patient is shown on top of the graph. These studies demonstrate decrease in the NCL-specific inclusions with the Cystagon treatment

Fig. 2 Disease severity scores (DSS) at different ages in the treated and control individuals. Average disease severity scores (DSS) are shown on the Y-axis and patients' age in years are shown on the X-axis. The treated patients are indicated with black lines and the controls with gray lines. Lower scores show more advanced

disease stage. (a) Results of all treated and control individuals combined. (b) Results in group 1. (c) Results in group 2. (d) Results in group 3. Only the results in group 1 show lower disease severity/progression in the Cystagon treated individuals (see text for further explanation)

parameters were added (maximum score of 18) to obtain the DSS. The DSS plotted against the patients' age are shown for the participants in each of the three observational

groups on Fig. 2. The combined scores of all study participants did not show any apparent difference in disease progression between treated and control individuals

(Fig. 2a). There was evidence of a slower disease progression in the two treated individuals in observation group 1 compared to nontreated controls (Fig. 2b). However, this slower disease progression was also observed in the treated individuals prior to the initiation of the treatment. In the other two observational groups, no treatment effect was noted as evidenced by the DSS. In addition, the parents of one of the patients from group 1 stated that they witnessed a decrease in the psychotic manifestations, auditory and visual hallucinations, and an improvement of the patient's gait and concentration paralleling the Cystagon administration. When the Cystagon was stopped for a brief period of time, the visual and auditory hallucinations returned. The parents also felt that the Cystagon administration improved the patient's overall functioning (Wen et al. 2012).

Limitations of the Study

Our study has a number of limitations. It involves a very small number of patients and it is not randomized. Also, the parents, who scored the patients' manifestations were not blinded regarding Cystagon administration. The scoring was done in a retrograde manner for the period prior the initiation of the study and some of the period of the study. All these limitations may have introduced a bias in the obtained results. The used DSS in its original development was validated only for patients between ages 3 and 20 (Kohlscutter et al. 1988). The scoring of our study participants was done from age 1. However, the Cystagon treatment was not started until after the third birthday in all treated patients.

Discussion

Various treatments for patients with *CLN1* mutations have been suggested, but no specific treatment was shown to be particularly successful to date. The cysteamine bitartrate (Cystagon) was shown to be able to metabolize the storage material in INCL. Study of phosphocysteamine, an agent with very similar structure and activity to Cystagon, was shown to prevent accumulation of storage material and to inhibit apoptosis in lymphoblasts of INCL patients (Zhang et al. 2001). However, later in vitro experiments with cysteamine showed inefficient cleavage of PTT substrates by this agent and by related aminothiols (Lu and Hofmann 2006). An ongoing trial using a combination of Cystagon and N-acetylcysteine in patients with typical *CLN1* mutations and infantile NCL, conducted in the National Institute of Child Health and Human Development, is listed on the NIH website "Clinicaltrials.gov", but per our knowledge no

results of this trial were published to date. No other human trials using Cystagon in NCL patients were reported/listed to date.

Our study showed that the use of Cystagon was associated with a decrease in the storage material in individuals with *CLN1* mutations. Such decrease seemed to be reversible if the Cystagon intake was discontinued or reduced. Cystagon use overall did not show significant attenuation in the disease progression if all treated patients were analyzed together. In one of the groups studied, the disease progression in the treated patients was slower compared to controls with the same *CLN1* mutations (observational group 1). However, this slower progression was seen prior to the initiation of the Cystagon treatment and may not have been related to the Cystagon administration. Interestingly, in observational group 1 the highest Cystagon dose was achieved. High Cysteamine doses were needed for a significant therapeutic effect in cystinosis (Gahl et al. 1987). Cysteamine was recently suggested as a possible treatment for Huntington disease. A new neuroprotection mechanism of Cysteamine was demonstrated, related to its effect in increasing the level of brain-derived neurotrophic factor in a model of Huntington disease (Borrell-Pages et al. 2006). Therefore, beneficial effects of the Cystagon in NCL other than substrate reduction are also a possibility.

In conclusion, overall no significant clinical positive effect of Cystagon was seen in the current study involving four patients. It is not clear if the slower disease progression in the treated patients in observational group 1 is partially due to Cystagon administration since this slower progression was observed prior to the initiation of the Cystagon treatment. It is possible that the small size of the study limited our ability to see small positive effect.

Acknowledgments This study is dedicated to the memory of Dr. Krystyna Wisniewski. The study was initiated and continued under her supervision until her death in 2008. Without her leadership, the study would not have been possible.

Synopsis

Cystagon treatment in patients with *CLN1* mutations.

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