Enzyme replacement therapy delays pupillary light reflex deficits in a canine model of late infantile neuronal ceroid lipofuscinosis

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A B S T R A C T

Late-infantile neuronal ceroid lipofuscinosis (CLN2 disease) is a hereditary neurological disorder characterized by progressive retinal degeneration and vision loss, cognitive and motor decline, seizures, and pronounced brain atrophy. This fatal pediatric disease is caused by mutations in the CLN2 gene which encodes the lysosomal enzyme tripeptidyl peptidase-1 (TPP1). Utilizing a TPP1 −/− Dachshund model of CLN2 disease, studies were conducted to assess the effects of TPP1 enzyme replacement administered directly to the CNS on disease progression. Recombinant human TPP1 (rhTPP1) or artificial cerebrospinal fluid vehicle was administered to CLN2-affected dogs via infusion into the CSF. Untreated and vehicle treated affected dogs exhibited progressive declines in pupillary light reflexes (PLRs) and electroretinographic (ERG) responses to light stimuli. Studies were undertaken to determine whether CSF administration of rhTPP1 alters progression of the PLR and ERG deficits in the canine model. rhTPP1 administration did not inhibit the decline in ERG responses, as rhTPP1 treated, vehicle treated, and untreated dogs all exhibited similar progressive and profound declines in ERG amplitudes. However, in some of the dogs treated with rhTPP1 there were substantial delays in the appearance and progression of PLR deficits compared with untreated or vehicle treated affected dogs. These findings indicate that CSF administration of TPP1 can attenuate functional impairment of neural pathways involved in mediating the PLR but does not prevent loss of retinal responses detectable with ERG.

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1. Introduction

Childhood-onset neuronal ceroid lipofuscinoses (NCLs) are autosomal recessively inherited lysosomal storage disorders characterized by progressive vision loss culminating in blindness, cognitive and motor decline, and seizures (Haltia and Goebel, 2012; Mole et al., 2011). The NCLs are almost always fatal. Clinical signs of NCL result from widespread, progressive neurodegeneration accompanied by accumulation of autofluorescent lysosomal storage bodies in CNS and other tissues, including the retina (Haltia, 2006). There are currently no effective treatments for any of the multiple forms of NCL, although enzyme replacement therapy for the CLN2 disease form is being tested clinically (ClinicalTrials.gov identifier: NCT01907087).

Development of effective therapies is facilitated by the availability of suitable animal models. Naturally occurring NCLs have been identified in several larger animal species, including dogs (Awano et al., 2006a, 2006b; Bond et al., 2013; Farias et al., 2011; Katz et al. 2005, 2011; O’Brien and Katz, 2008; Palmer et al., 2011; Sanders et al., 2010). Among the canine NCLs, long-haired Dachshunds have a null mutation in TPP1 which encodes the lysosomal enzyme tripeptidyl-peptidase-1 (TPP1) (Awano et al., 2006b). Neurological signs in children with mutations in CLN2 (the human ortholog of TPP1) typically appear between 2 and 4 years of age. Affected children suffer from progressive vision loss and profound progressive neurological decline. The neurological deterioration and accompanying brain atrophy associated with CLN2 disease ultimately leads to death, usually by the middle
teenage years (Haltia and Goebel, 2012; Mole et al., 2011). Dachshunds homozygous for a TPP1 null mutation develop neurological signs and vision loss that recapitulates the disease progression observed in children with CLN2 disease and reach end stage disease between 10 and 11 months of age (Awano et al., 2006b; Katz et al., 2008; Sanders et al., 2011; Vuillemenot et al., 2011).

TPP1 is a soluble lysosomal enzyme that plays an important role in protein catabolism. Like other soluble lysosomal enzymes, TPP1 is not only localized within lysosomes, but is also discharged from cells and taken back up via cell surface receptors that recognize the mannose 6-phosphate moieties common to many lysosomal enzymes. TPP1 is synthesized as a glycosylated proenzyme that is activated by proteolytic cleavage of an N-terminal fragment after incorporation into lysosomes (Guhaniyogi et al., 2009). TPP1 proenzyme supplied exogenously to cells is taken up via cell surface mannose 6-phosphate receptors and is transported via the endosomal system to lysosomes where it is activated (Kytälä et al., 2006; Sohar et al., 1999). Therefore, CLN2 disease is amenable to TPP1 enzyme replacement therapy (ERT). Because large molecules such as TPP1 cannot cross the blood–brain barrier, delivery of the enzyme to the brain has been achieved through administration of TPP1 pro-enzyme by infusion into the cerebrospinal fluid (CSF) in mouse and dog (Chang et al., 2008; Vuillemenot et al., 2011). In the Dachshund CLN2 disease model, we have previously shown that this route of TPP1 administration results in widespread distribution of the active enzyme in many structures of the brain and in reduction in the accumulation of neuronal lysosomal storage material that is characteristic of this disease (Vuillemenot et al., 2011). In addition, biweekly enzyme infusions delayed the onset and progression of neurologic signs and brain atrophy, improved cognitive function, and substantially extended lifespan in the Dachshund model (Katz et al., 2014).

If TPP1 infused into the CSF can reach the tissues involved in retinal-mediated light responses, this treatment may preserve visual function as well. Quantitative evaluation of the pupillary light reflex (PLR) in conjunction with electroretinogram (ERG) assessment is a sensitive tool to evaluate the integrity of the entire complex network of neuronal circuitry involved in modulating pupal pupil size, including the retina from which the signals originate that generate the PLR (Fotiou et al., 2000; Park et al., 2011; Whiting et al., 2013a). Utilizing the PLR in conjunction with the ERG can be particularly useful in characterizing diseases such as CLN2 in which pathological changes occur in both the retina and other areas of the CNS involved in mediating the PLR and in assessing the pharmacological activity of therapeutic interventions.

Dogs affected with CLN2 disease present with marked decline in retinal function as demonstrated by reduced ERG b-wave amplitudes by 5 months of age and significant thinning of the inner retina by disease end-stage (Katz et al., 2008; Whiting et al., 2013a). In addition, affected dogs exhibit significant deficits in PLR latency, constriction velocity, constriction amplitude, and both initial and secondary redilation velocity, particularly with dim light stimuli (Whiting et al., 2013a). We hypothesized that these PLR deficits result not only from retinal degeneration but also from disease-related degenerative changes in the CNS nuclei involved in mediating the PLR and that providing rhTPP1 to the CNS would inhibit development of these deficits. Studies were therefore undertaken to determine whether chronic administration of pro-rhTPP1 into the CSF could ameliorate PLR deficits associated with CLN2 disease.

2. Materials and methods

2.1. Animals

Studies employed long-haired miniature Dachshunds bred and housed in a research facility at the University of Missouri. A research colony was established by breeding from an original pair of Dachshunds that were heterozygous for a one nucleotide deletion in TPP1 (Awano et al., 2006b). Puppies were genotyped within several weeks of birth at the TPP1 locus using an allelic discrimination assay that distinguishes the normal and mutant alleles (Awano et al., 2006b). Most breeding consisted of carrier to carrier crosses, but periodically carrier males were bred to unrelated normal females to maintain genetic heterogeneity in the colony. Dogs utilized in this study were either homozygous normal (TPP1+/+) (n = 16) or homozygous for the mutant allele (TPP1−−) (n = 16). Heterozygous carriers of the TPP1 mutation (TPP1+/−) were not evaluated for responses to light stimuli. Dogs were entrained to a 12:12 daily light cycle and were socialized daily in addition to receiving routine husbandry care. All studies were performed in compliance with the EU Directive 2010/63/EU for animal experiments and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Missouri Animal Care and Use Committee.

2.2. Ophthalmic examinations

Prior to inclusion in the study, all dogs received a complete ophthalmic examination at 10–12 weeks of age, before the onset of any signs of retinal or neurological impairment due to CLN2 disease (Katz et al., 2008; Whiting et al., 2013a). Any dogs with evidence of vision compromise or ophthalmic conditions deemed threatening to vision were excluded from the study (n = 1). Examinations were repeated monthly for CLN2-affected dogs and every other month for genetically normal dogs. Examinations included assessment of visual tracking behavior, slit lamp biomicroscopy, and indirect ophthalmoscopy. Slit lamp biomicroscopy was performed prior to dilation (SL14; Kowa Co. Ltd., Tokyo, Japan). Pupils were dilated with a short-acting mydriatic (tropicamide 1%; Alcon, Fort Worth, TX) prior to indirect ophthalmoscopy which was performed using a wireless indirect headset (12,500, Welch Allyn Inc., Skaneateles Falls, NY, USA) and a handheld lens (30 diopter clear lens, Volk Optical Inc., Mentor, OH). Fundus photographs were taken following examination (NM-100; Nidek Co. Ltd., Freemont, CA) and archived electronically. Photos were reviewed to evaluate for changes in retinal appearance.

2.3. Delivery of replacement enzyme

Recombinant human pro-TPP1 (rhTPP1) was synthesized and purified as described previously and formulated in artificial CSF (Lin and Lobel, 2001). Artificial CSF (vehicle) was administered alone to vehicle control dogs. At approximately 2 months of age, two catheters were implanted in each dog. One catheter terminated in a lateral ventricle of the brain (ICV catheter) and the other in subarachnoid

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>n</th>
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<tbody>
<tr>
<td>Normal</td>
<td>TPP1+/+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Vehicle Treated</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Enzyme Treated: 16 mg rhTPP1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Enzyme Treated: 4 mg rhTPP1</td>
<td>3</td>
</tr>
<tr>
<td>Affected</td>
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</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Vehicle Treated</td>
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<td>Enzyme Treated: 4 mg rhTPP1</td>
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<td>Enzyme Treated: 48 mg rhTPP1</td>
<td>2</td>
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</tbody>
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space at L5 (ITL catheter). The catheters were connected to subcutaneous titanium access ports anchored in the fascia of the respective muscle and subcutaneous tissues of the cervical and lumbar regions. Detailed descriptions of the catheter and port implantation procedures are provided in the Supplemental Materials section.

Starting 2 weeks after the catheter implantation surgeries, test or control articles were administered via infusion through the ICV catheters once every other week (Table 1). Each infusion was performed over a 2–4 h period, depending on dose. The 4 mg doses were administered over 2 h infusion periods and the higher doses were administered over 4 h infusion periods. The dogs were sedated with buprenorphine (0.015 mg/kg) and dexmedetomidine (30–40 mcg/kg). The treatment groups are listed in Table 1.

After 4 months of age, prior to each infusion, the location within the lateral ventricle and patency of the ICV catheter was assessed by computed tomography of the brain after infusion of 0.1 ml of the nonionic contrast agent Iohexol (GE Healthcare). Over time, the ICV catheter in most animals either became occluded or the tip migrated into the brain parenchyma due to growth of the cranial cavity. When this occurred, the infusion site was switched from the ICV to the TFL catheter. Eventually, the TFL catheters usually became occluded as well. When this occurred, the treatment administration was continued by bolus injection into the subarachnoid space at the cerebellomedullary cistern over 2 min.

2.4. PLR recordings

The PLR was recorded in each dog at 8, 10 and 12 months of age. PLR recordings were also obtained at 15 months of age in two treated, affected dogs that received the 16 and 48 mg doses and three normal dogs that were either untreated (n = 2) or received the 16 mg dose (n = 1). Due to the need for euthanasia prior to the recording time point, the 10 month recording was not obtained in two of the vehicle treated dogs. For the same reason, the 12 month recording was not obtained in two of the treated, affected dogs (4 mg rhTPP1 (n = 1); 48 mg rhTPP1 (n = 1)) or any of the untreated or vehicle treated affected dogs. All recordings were done during the light period of the daily 12:12 light–dark cycle. The detailed methods for obtaining the PLRs have been described previously (Whiting et al., 2013b). Dogs were kept in dim light (0.9 lux) for at least 1 h, including preparation time, and in complete darkness for 10 min prior to recording. After 30 min of dim light adaptation, dogs were pre-medicated with dexmedetomidine (20–25 μg/kg IM) prior to induction of anesthesia with propofol [IV to effect, 1.49 ± 0.59 mg/kg (mean ± SD); PropoFlo 28, Abbott Laboratories, Abbott Park, IL]. Dogs were intubated with a cuffed endotracheal tube and anesthesia maintained with isoflurane (1.5% vaporizer setting; Terrell, Piramal Healthcare, Boise, ID) in oxygen. A lid speculum was inserted to ensure that the nictitating membrane and eyelids did not interfere with light exposure or visualization of the pupil. In addition, a small stay suture was placed in the bulbar conjunctiva on the central axis approximately 5 mm superior to the limbus to facilitate globe manipulation to maintain centration of the pupil on the optical axis of the recording apparatus. The eye was regularly lubricated with saline eye wash solution throughout the procedure.

Unilateral recordings were performed with a custom apparatus (Whiting et al., 2013a; Fan et al., 2009) capable of timed delivery of a visible light stimulus from a mounted high-power broad spectrum LED (MCHWL2; Thorlabs Inc., Newton, NJ) and concurrent recording of pupil images at 30 frames per second using an infrared-sensitive camera (PC164CEX-2; Supercircuits Inc., Austin, TX) and continuous infrared illumination (880 nm LED) for visualization of the eye. The direct PLR of the right eye was evaluated using a standardized protocol of 100 ms flashes of broad spectrum white light at each of 10 intensities between 8 and 15 log photons/cm²/s.

Pupil images were analyzed using the batch processing feature in Photoshop (Adobe Systems Inc; San Jose, CA). A list of image frame number and corresponding pupil area was exported to a spreadsheet and used to calculate desired parameters. Area measurements were converted from pixels to mm² based on the known size of the lid speculum present in each pupil image (Whiting et al., 2013b).

2.5. PLR parameters

For the studies described here, baseline pupil area was the average pupil area, in a dark-adapted dog, over a 1 s period before the light stimulus. Baseline pupil diameter was calculated from area measurements of the circular pupil, PLR constriction amplitude was defined as the difference between baseline pupil area and minimum pupil area attained following the light stimulus. These values were then converted to a percentage of baseline pupil area. Latency was defined as the time between stimulus onset and the beginning of pupil constriction. Average constriction velocity was calculated as the constriction amplitude divided by the constriction time, where constriction time is calculated between the beginning of pupil constriction and the minimum pupil size.

Redilation of the pupil is biphasic with a fast initial redilation and slower secondary redilation. Average redilation velocity was calculated for the initial redilation phase as half the constriction amplitude divided by the time required for the pupil to redilate from its minimum size to half the baseline pupil size. For the brightest flash (15 log photons/cm²/s), the average rate of secondary redilation was calculated for the period from 15 to 85 s after light offset.

2.6. Electoretinography

Beginning at 2 months of age, bilateral ERG evaluations were performed monthly as previously described (Katz et al., 2008). For ages at which dogs underwent PLR assessment, ERG evaluation was performed within one week after the PLR recording session. Dogs were prepared for ERG recording in ordinary room light. Prior to recording, both pupils were dilated with 1% tropicamide, and dogs were deeply sedated with intramuscular administration of dexmedetomidine (30–40 μg/kg). Ketamine (up to 5 mg/kg) was used in combination with the dexmedetomidine for dogs 6 months of age and younger to achieve adequate sedation. It was omitted in older dogs to reduce the risk of seizure associated with CLN2, and it was no longer necessary for adequate sedation with this age group.

ERGs were bilaterally elicited and simultaneously recorded with a portable unit (HMSERG model 2000; RetVet Corp., Columbia, MO). The right and left mini-ganzfeld domes were positioned approximately 2 cm from the corresponding eye. Each ERG session consisted of scotopic and photopic recordings in accordance with the Dog Diagnostic Protocol, recommended by the European College of Veterinary Ophthalmology, primarily for evaluation of rod and cone function (Narfström et al., 2002). Throughout a 20 min period of dark adaptation, scotopic low-intensity rod responses were elicited at a stimulus intensity of 10.2 log photons/cm²/s (0.01 cd/m²) with 4 min of dark adaptation between recordings. Thereafter, scotopic responses were elicited using flashes of 12.65 and 13.2 log photons/cm²/s (3 cd/m² and 10 cd/m²) to evaluate mixed rod and cone function. The eyes were then exposed to diffuse white light at a luminance of 13.65 log photons/cm²/s (30 cd/m²) for 10 min, immediately after which responses to single 12.65 log photons/cm²/s (3 cd/m²) flash stimuli were recorded. This was immediately...
followed by evaluation of responses to 30-Hz photopic flicker stimuli at the same light intensity. ERG waveforms in all recordings were evaluated, and the amplitudes and implicit times for the a- and b-waves were measured as previously described (Marmor et al., 2004).

2.7. Optic nerve axon number determinations

Affected Dachshunds were euthanized at end stage disease and related homozygous normal Dachshunds were euthanized at similar ages (11–15 months). Immediately after euthanasia the eyes were enucleated and an approximately 10 mm long segment of the optic nerve was dissected from immediately behind the globe and fixed in 2% glutaraldehyde, 1.12% paraformaldehyde, 130 mM sodium cacodylate, 1 mM CaCl2 at pH 7.4. Approximately 3 mm long segments of the optic nerves were post-fixed in osmium tetroxide and embedded in epoxy resin. Cross sections of these samples were cut at a thickness of 0.4 μm, mounted on slides and stained with toluidine blue. Composite high resolution images of the entire cross section of each optic nerve were obtained as described previously (Morgan et al., 2014) (Fig. 1). Axon numbers in two size classes (greater than and less than 7.8 μm2 in cross-sectional area) were determined using Adobe Photoshop Touch and Metamorph (Molecular Devices, Sunnyvale, CA).

2.8. Statistical analysis

All statistical tests were performed using SigmaPlot (Systat Software Inc., San Jose, CA). Data were subjected to the Shapiro-Wilk test to confirm normal distribution. Results from all genetically normal dogs (TPP1+/+), including those that were left untreated, those that received infusions of vehicle, and those that received infusions of rhTPP1 were combined to serve as the normal control group. Results from CLN2-affected dogs that received infusions of vehicle were combined with those from untreated, affected dogs to serve as the disease control group. Results from CLN2-affected dogs that received infusions of vehicle were combined with those from untreated, affected dogs to serve as the disease control group. Pairwise comparisons did not detect any significant differences in any of the parameters that were measured between groups that were combined for the final statistical analyses.

For the ERG and PLR data, repeated measures 2-way ANOVA was used to compare results from affected dogs treated with rhTPP1 to each of these control groups to determine if enzyme replacement therapy was able to normalize any PLR or ERG deficits related to CLN2 disease progression. Follow-up pairwise comparisons were performed with the Holm-Sidak correction (α = 0.05) to control family-wise error rate. For the optic nerve axon count data, comparisons between untreated, affected and normal dogs were performed using Student’s t-test. Because no significant effect of the disease alone on optic nerve axon number was detected (see results), no attempt was made to assess whether there was an effect of rhTPP1 treatment on this parameter.

3. Results

3.1. Ophthalmic examinations

Initial examination in all dogs revealed normal adnexal and ocular structures. Some CLN2-affected (TPP1−/−) dogs in each of the treatment groups developed progressive, multifocal retinal detachment lesions between the ages of 5 and 11 months (n = 11). These disease-related lesions have been described previously (Pearce et al., 2012). The lesions did not significantly alter the PLR or ERG except in advanced cases affecting over 50% of the retina (Whiting et al., unpublished data), which occurred in one of the untreated dogs at 10 months of age. In order to avoid confounding factors, the data collected when severe lesions were present were excluded from the study. Other CLN2-affected dogs retained phenotypically normal fundus exams throughout the study period (n = 6). Homozygous normal (TPP1+/+) dogs had consistently normal ophthalmic examinations throughout the study period.

3.2. PLR

CLN2-affected dogs that were left untreated and vehicle controls exhibited significantly reduced PLR constriction amplitudes by 8 months of age with stimuli between 9 and 12 log photons/cm²/s (Figs. 2 and 3). These deficits were even more pronounced by 10
months of age (Figs. 2 and 4). Among the treated dogs, there was variability in the treatment effect that did not depend on treatment dose level (Table 2). Of the eight dogs treated with rhTPP1, three dogs (4 mg rhTPP1 (n = 2); 16 mg rhTPP1 (n = 1)) retained normal PLR constriction amplitude through 10 months of age indicating a response to treatment (Group R) (Table 2; Fig. 2). In the remaining treated dogs, however, treatment did not preserve the PLR (Group NR) and the PLR changes mimicked the deficits in constriction amplitude observed in untreated, affected dogs at both 8 and 10 months of age (Table 2; Figs. 3 and 4). These trends were also observed for other PLR parameters with Group R treated dogs exhibiting normal latency, constriction velocity, and initial redilation velocity, particularly with low stimulus intensities (Fig. 5). (Whiting et al., 2013a, 2013b). Group NR treated dogs, however, exhibited deficits in each of these parameters characteristic of untreated CLN2 dogs (Whiting et al., 2013a).

Sample size decreased with age because of the need for euthanasia prior to the 10, 12, and 15 month recording time points due to the symptoms associated with end-stage disease. A single untreated dog was removed from the study prior to the 10 month recording due to the development of severe retinal lesions.

By 12 months of age, enzyme treated, CLN2-affected dogs from Group R that initially exhibited normal PLR constriction amplitudes, began to show PLR deficits indicating a waning effect of the treatment (Fig. 6). However, constriction amplitudes in Group R were still significantly increased over those of dogs that did not respond to treatment (Group NR) for stimuli of 9 and 10 log photons/cm²/s (p < 0.05).

Deficits in PLR constriction amplitude at 15 months of age (Fig. 7) were very similar for both dogs treated with rhTPP1 that survived to this age, which included one dog that initially responded to treatment and one dog that did not respond to treatment. However, the change between 12 months and 15 months of age was much more pronounced in the dog that responded to treatment and initially retained normal PLR parameters (Fig. 7). PLR threshold at 15 months of age was increased by over 3 log units from the average threshold for this group (R) at 12 months of age. For the 15 month old dog that initially responded to treatment, these PLR changes coincided with end-stage disease. However, the 15 month old dog from the non-responding group (NR) had only begun to show neurologic signs of disease. Both 15 month old dogs had severe deficits in inner retinal function as illustrated by reduction of the ERG b-wave amplitude.

Analysis of sustained pupil size after a bright stimulus (15 log photons/cm²/s) revealed a significant delay (p < 0.05) in secondary redilation of the pupil to baseline size in rhTPP1 treated CLN2-affected dogs (Fig. 8), similar to deficits found in untreated dogs (Whiting et al., 2013a). This deficit in secondary redilation was present for all CLN2-affected dogs, even those treated with rhTPP1 enzyme that exhibited normal values for other PLR parameters including constriction amplitude, latency, constriction velocity, and initial redilation velocity (Group R). The rate of secondary redilation was significantly reduced in treated, affected dogs compared with genetically normal dogs between 8 and 12 months of age, indicating that post-illumination pupil constriction persisted longer after light offset in these dogs than in normal dogs.

3.3. ERG

Deficits in both scotopic and photopic ERG b-wave amplitudes of CLN2-affected dogs that were treated with rhTPP1 at any dose level were very similar to those of untreated, affected dogs (Fig. 9). CLN2-affected, treated dogs from group R that exhibited normal PLR parameters show no improvement in b-wave amplitude compared with either untreated and vehicle treated dogs or treated dogs that showed no improvement in PLR parameters (group NR).
3.4. Optic nerve axon numbers and size

Although optic nerve samples from all dogs were prepared using the same methods, the quality of fixation required to obtain reliable total axon counts was only adequate in 5 of the normal, 3 of the vehicle-treated affected dogs, and 2 of the TPP1-treated affected dogs (the latter 2 samples were not included in the quantitative analyses). No significant differences were observed between the vehicle-treated, affected and normal dogs for either total axon numbers or in the distribution of axon sizes between the large and small categories (Table 3). Due to the variance within each group, the statistical power of this comparison was low (0.11). It may have been possible to detect a disease-related loss of ganglion cells had a larger number of dogs been evaluated. Power analyses indicated that based on the variance of the data within each group the number of dogs that would have been required to detect a 10% difference in mean axon numbers was 13 dogs per group with a statistical power of 0.8 and an alpha of 0.05 (assuming the larger number of dogs reduced the standard deviation within each group to 13,000). Clearly if this many dogs were required to detect a 10% difference in axon numbers, any such difference is not meaningful with regard to disease mechanism or treatment effects.

4. Discussion

rhTPP1 ERT administered directly to the brain via the CSF was able to delay PLR deficits associated with CLN2 disease in some dogs, despite the fact that these treatments did not inhibit the decline in retinal function detectable with ERG. CLN2-affected dogs that showed treatment effects of enzyme replacement displayed normal PLR constriction amplitude through 10 months of age and didn’t begin to display deficits until 12 months of age. This is a substantial improvement relative to the untreated and vehicle treated, affected dogs which began to exhibit deficits by 6 months of age that progressed through end-stage disease at 10 months of age.

The PLR depends on the function of both the retina and specific nuclei in the midbrain. All of these tissues exhibit an accumulation of disease-related storage material in CLN2-affected dogs, indicating that TPP1 function is important in both the retina and in these areas of the CNS (Katz et al., 2008; Whiting et al., 2013a). Based on the CSF circulation, the only retinal neurons expected to possibly take up rhTPP1 infused into the CSF are the ganglion cells, which may be able to take up the enzyme from the CSF circulating

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Table 2
Treatment group sample sizes for PLR & ERG recordings with CLN2-affected dogs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment administered</th>
<th>n for given age in months:</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (Response to treatment)</td>
<td>4 mg rhTPP1</td>
<td>2 2 1 1</td>
</tr>
<tr>
<td></td>
<td>16 mg rhTPP1</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>NR (No response to treatment)</td>
<td>4 mg rhTPP1</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>16 mg rhTPP1</td>
<td>2 2 1 1</td>
</tr>
<tr>
<td></td>
<td>48 mg rhTPP1</td>
<td>2 2 1 1</td>
</tr>
<tr>
<td>Diseased control</td>
<td>Untreated</td>
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</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>3 1 1 1</td>
</tr>
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Fig. 5. PLR Parameters from 10 month old dogs (mean ± SEM) including latency (A), average constriction velocity (B), and average redilation velocity (C). Treated dogs from Group R (n = 3) showed a response to treatment and exhibited PLR parameters that are significantly different from those of vehicle treated and untreated, CLN2-affected dogs (n = 5) (*, p < 0.05).

Fig. 6. PLR constriction amplitudes from 12 month old dogs (mean ± SEM). Amplitudes are reduced from normal in CLN2-affected dogs which initially responded to treatment (group R; n = 2) and exhibited normal PLR parameters. The development of these deficits indicates a waning treatment effect. However, constriction amplitudes in this group are still significantly greater (†, p < 0.05) than those of the dogs that exhibited no response to enzyme treatment (NR; n = 4) for stimuli of 9 and 10 log photons/cm²/s. None of the affected, untreated or vehicle treated dogs lived to be 12 months of age.
through the meninges ensheathing the optic nerve. However, based on the optic nerve axon count data, it does not appear that the disease results in loss of retinal ganglion cells, so the effect of the treatment in preserving the PLR in some dogs is not due to prevention of ganglion cell death, regardless of whether TPP1 got to these cells via the CSF. The disease also had no effect on the size distribution of the optic nerve axons, indicating that NCL did not result in swelling or shrinkage of these axons. It is unlikely that significant TPP1 was able to reach the cells of the inner retina, which undergo severe degeneration in CLN2 disease (Katz et al., 2008). Consistent with this expectation, rhTPP1 administered via the CSF did not result in any improvement in the ERG b-wave, which reflects activity of retinal neurons with cell bodies located in the retinal inner nuclear layer. Based on these data, it seems likely that the infused enzyme was able to normalize the PLR primarily by preserving the function of CNS neurons involved in mediating the PLR including the pretectal and oculomotor nuclei. Although the disease-related decline in the PLR was not associated with ganglion cell loss, it is possible that CLN2 disease affected ganglion cell function, including the function of intrinsically photosensitive retinal ganglion cells (ipRGCs) that are involved in mediating the PLR but do not contribute to the ERG (Pickard and Sollars, 2012).

It is possible that preservation of the PLR by rhTPP1 administration in some of the dogs resulted from uptake of the enzyme from the CSF by the brain and spinal cord neurons involved in mediating the PLR. Indeed, we previously demonstrated that infusion of rhTPP1 into the CSF resulted in widespread uptake of active TPP1 enzyme into many brain regions (Vuillemenot et al., 2011), including the brain nuclei involved in the PLR (unpublished data). In untreated affected dogs there is widespread progressive brain atrophy that likely includes loss of or at least functional impairment of the central nervous system neurons involved in the PLR. Restoration of TPP1 enzyme to these cells is likely to preserve their function and survival.

Even in dogs that demonstrated a preservation of the PLR in response to rhTPP1 infusions, the treatment effect was temporary, and declines in the PLR responses continued with advancing age. This suggests that CSF administration may have only partially restored intracellular TPP1 activity levels in neurons in the PLR pathway. The result may have been a slowing in the rate of progression of disease pathology at the cellular level, but not a complete prevention. Optimizing delivery of functional TPP1 enzyme throughout the brain and delivering the enzyme to the retina as well may result in more prolonged or even complete preservation of the PLR.

The efficacy of CSF administration of rhTPP1 in preserving the PLR was variable, with only one-third of affected dogs that were treated exhibiting PLR preservation. This may be due to long term variability in the route of rhTPP1 administration, as the number of infusions for each route (IVC, ITL, bolus injection) differed for each dog. In addition, the variability in pharmacological effect may have been due in part to differences in the distribution of rhTPP1 due to variations in CSF flow and enzyme uptake by different brain structures. Such variability would be expected based on the known flow dynamics of CSF. Quantitative assessments indicated that at the time of euthanasia, there was substantial variability in TPP1 levels in the same brain regions between dogs that had received the same dose of rhTPP1 (unpublished data).

The fact that some rhTPP1 treated dogs did display PLR preservation indicates that this effect is possible with CSF administration of rhTPP1. In order to optimize the treatment it will be important to identify the variables that determine efficacy so that preservation of the PLR, as well as of other neurological functions, can be achieved consistently. Although the critical variables that determined efficacy could not be identified based on the current study, there was no correlation between rhTPP1 dose and PLR preservation, so it seems unlikely that simply increasing the amount of therapeutic protein administered would enhance the treatment effect relative to preserving the PLR.

Both treated and untreated, CLN2-affected dogs exhibited delays in the secondary redilation phase of the PLR. Melanopsin input to the PLR is responsible for the prolonged constriction typical after offset of a sufficiently bright stimulus (Kankipati et al., 2010; Markwell et al., 2010; Dacey et al., 2005). It is possible that the reduced rate of secondary redilation in older affected dogs may reflect altered sympathetic input to the iris, and/or altered effect on the ipRGCs from OFF-bipolar and amacrine cells which both provide inhibitory input (Belenky et al., 2003; Brown and Lucas, 2009). The slower secondary post-illumination redilation seen in affected dogs is consistent with an up regulation of the melanopsin-driven intrinsic photosensitivity as a consequence of a reduction in outer retinal inputs as evidenced by the reduction in the b-wave of the ERG. rhTPP1 was unable to normalize this phase of the PLR despite
the improvement observed in other PLR parameters. Since rhTPP1 did not appear to attenuate retinal degeneration, this may indicate a lack of retinal inhibition as the source of these redilation abnormalities. However, due to the variable distribution of rhTPP1 when administered via the CSF, it is possible that the central and peripheral sympathetic neurons involved in the slow redilation phase of the PLR did not receive therapeutic amounts of TPP1 in some dogs, and degeneration of these areas contributed to the delays in secondary redilation.

The timing of changes in the PLR associated with CLN2 disease did not coincide closely with changes in ERG responses or with neurologic signs caused by neuronal degeneration. In untreated and vehicle treated dogs, mild PLR changes occur early in the disease at 6–8 months of age, which coincides with the most rapidly progressing deficits in the ERG. However, no large changes occur in the ERG after 8 months of age while the most extreme PLR deficits occur at 10 months of age and coincide with the appearance of severe neurologic deficits, thus supporting CNS disease. In dogs treated with ERT, the timing of declines in ERG responses was not altered. These findings suggest that alterations in central neural pathways play a predominant role in the development of PLR deficits in canine CLN2. However, the lag between the declines in the PLR relative to those in the ERG may also be due in part to the significantly lower threshold of the PLR compared with the ERG (Whiting et al., 2013b). There is likely a complicated interaction of deficits in the retina and other CNS areas involved in modulating the PLR. Further analysis of these tissues at various disease stages would be needed to fully explain the mechanisms involved in PLR decline and the efficacy of ERT in preserving the PLR in some dogs despite profound ERG deficits.

The inability of CSF-administered rhTPP1 to preserve the ERG indicates that while this treatment may have profound benefits in inhibiting the progression of many neurological deficits (Katz et al., 2013) it is unlikely to preserve vision. However, like the rest of the nervous system, retinal cells should be able to take up TPP1 from their close environment and incorporate it in active form into their lysosomes. Therefore, in order to preserve vision in CLN2, it may be necessary to restore TPP1 activity in the eye via ERT directly. If
current clinical trials of ICV administered rhTPP1 are successful in attenuating neurodegeneration, administration directly to the ocular tissues may be indicated.

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Appendix A. Supplementary data

Supplementary data related to this chapter can be found at http://dx.doi.org/10.1016/j.exer.2014.06.008.

References


