Experimental Eye Research 152 (2016) 77–87

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

Intravitreal implantation of *TPP1*-transduced stem cells delays retinal degeneration in canine CLN2 neuronal ceroid lipofuscinosis

Christopher J. Tracy [a, b, 1], Rebecca E.H. Whiting [a, 1], Jacqueline W. Pearce [c], Baye G. Williamson [c], Daniella P. Vansteenkiste [c], Lauren E. Gillespie [a], Leilani J. Castaner [a], Jeffrey N. Bryan [c], Joan R. Coates [c], Cheryl A. Jensen [a], Martin L. Katz [a, b, *]

[a] Department of Ophthalmology, School of Medicine, University of Missouri, Columbia, USA
[b] Genetics Area Program, University of Missouri, Columbia, USA
[c] Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, USA

ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form
8 September 2016
Accepted in revised form 12 September 2016
Available online 13 September 2016

Keywords:
Retinal degeneration
Gene therapy
Stem cell therapy
Dog model
Electroretinogram
Retinal detachment

ABSTRACT

The CLN2 form of neuronal ceroid lipofuscinosis is a neurodegenerative disease that results from mutations in the *TPP1* gene. Affected children exhibit progressive declines in most neurological functions including vision. Functional declines are accompanied by progressive brain and retinal atrophy. *TPP1* encodes the soluble lysosomal enzyme tripeptidyl peptidase-1 (TPP1). Dachshunds with a *TPP1* null mutation exhibit a disorder very similar to human CLN2 disease. Periodic infusion of recombinant TPP1 protein or a single injection of a *TPP1* gene therapy vector into the cerebrospinal fluid of affected dogs significantly delays the onset and progression of neurological signs but does not slow vision loss or retinal degeneration. Studies were conducted to determine whether intravitreal implantation of autologous bone marrow derived stem cells transduced with a TPP1 expression construct would inhibit retinal degeneration in the canine model. A single injection of the transduced cells at an early stage in the disease progression substantially inhibited the development of disease-related retinal function deficits and structural changes. No adverse effects of the treatment were detected. These findings indicate that *ex vivo* gene therapy using autologous stem cells is an effective means of achieving sustained delivery of therapeutic compounds to tissues such as the retina for which systemic administration would be ineffective.

1. Introduction

The CLN2 form of neuronal ceroid lipofuscinosis (NCL) is an inherited, autosomal recessive lysosomal storage disease characterized by progressive neurological decline and vision loss. CLN2 disease results from mutations in the *TPP1* gene, which encodes the soluble lysosomal enzyme tripeptidyl peptidase-1 (TPP1) (Sleat et al., 1997). Deficiencies in this enzyme result in clinical signs that include seizures, loss of motor function, ataxia, dementia, impaired swallowing and respiration as well as progressive vision loss culminating in blindness. Death usually occurs by early to mid-adolescence due primarily to the secondary effects of severe neurological impairment (Andermann et al., 1988; Caraballo et al., 2005; Mole et al., 2011; Taratuto et al., 1995). Diseases that result from deficiencies in soluble lysosomal enzymes are amenable to treatments in which a functional version of the enzyme is administered to the affected individual and is taken up via normal cellular lysosomal transport mechanisms into the lysosomes of the target cells where it then performs its normal function (Sands and Davidson, 2006). Dachshunds with a naturally occurring null mutation in *TPP1* have been established as model for CLN2 disease (Awano et al., 2006; Katz et al., 2008, 2014; Sanders et al., 2011; Whiting et al., 2013, 2015). Previous studies with the canine model have demonstrated that periodic infusion of recombinant TPP1 into the cerebrospinal fluid (CSF) or transduction of cells

* Corresponding author. Department of Ophthalmology, School of Medicine, University of Missouri, One Hospital Drive, Columbia, MO 65212, USA.
E-mail addresses: cjtrvd@mail.missouri.edu (C.J. Tracy), WhitingRE@health.missouri.edu (R.E.H. Whiting), pearcej@missouri.edu (J.W. Pearce), baye_williamson@yahoo.com (B.G. Williamson), vansteenkisted@missouri.edu (D.P. Vansteenkiste), legc87@mail.missouri.edu (L.E. Gillespie), castanerl@missouri.edu (L.J. Castaner), bryanjn@missouri.edu (J.N. Bryan), jensenc@health.missouri.edu (C.A. Jensen), katzm@health.missouri.edu (M.L. Katz).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.exer.2016.09.003

lining the brain ventricles with a TPP1 expression vector are effective in delaying the onset and progression of neurological disease signs that result from the underlying enzyme deficiency (Katz et al., 2014, 2015; Vuillemenot et al., 2011; Vuillemenot et al., 2015; Whiting et al., 2014). However, these treatments did not inhibit the progressive decline in electrophysiological responses of the retina to light stimuli because the CSF, and therefore TPP1 within the CSF, does not contact any part of the retina other than the axons of the retinal ganglion cells. Therefore, effective enzyme replacement therapy for the retina in CLN2 disease will likely require direct and sustained delivery of the TPP1 protein to the retina.

The use of repeated intravitreal injections for administration of therapeutic agents to the retina can be effective for treating degenerative eye diseases but is far from ideal (Colucciello, 2015; Dedania and Bakri, 2015; Gardlik and Fusekova, 2015; Modi et al., 2015; Ramsey et al., 2014; Schwartz et al., 2014; Shikari et al., 2014; Song and Xia, 2015). Intraocular injections are associated with risks for injury and inflammation, and repeated injections may lead to formation of scar tissue at the injection sites. In addition, the cumulative costs of such treatments are high. Direct administration of a TPP1 gene therapy vector to the retina is a possible alternative method of achieving long-term sustained delivery of the TPP1 protein to the retina. However, inducing high levels of TPP1 synthesis and secretion by the highly specialized cells that make up the retina would entail the risk of disrupting normal retinal function by upsetting the precise intracellular homeostasis and intercellular interactions that are required to maintain normal retinal function. An alternative approach for long-term intraocular delivery of therapeutic agents is to implant transgenic cells into the vitreous that would continuously produce and release therapeutic agents. In the case of CLN2 disease, studies were undertaken to evaluate the therapeutic efficacy of an *ex vivo* gene therapy approach using genetically modified autologous mesenchymal stem cells for sustained delivery of TPP1 to the retina using the Dachshund model.

2. Materials and methods

2.1. Animals

A research colony of miniature long-haired Dachshunds was established by breeding from an original pair of Dachshunds that were heterozygous for a one nucleotide deletion (c.325delC) in exon 4 of *TPP1* (Awano et al., 2006). The mutation results in a frame shift and a complete absence of TPP1 enzymatic activity in dogs that are homozygous for the mutant allele. Dogs that are homozygous for the mutant allele suffer from a progressive neurodegenerative disease analogous to the CLN2 form of human late-infantile neuronal ceroid lipofuscinosis (Awano et al., 2006; Katz et al., 2008, 2014, 2015; Vuillemenot et al., 2011). Among the signs exhibited by the affected dogs is progressive retinal degeneration accompanied by loss of retinal function (Katz et al., 2008; Whiting et al., 2013, 2015). Heterozygous dogs are phenotypically normal. Breedings consisted of carrier to carrier crosses and female carrier to affected male crosses. To maintain background genetic heterogeneity, periodically carrier males were bred to unrelated normal females from various sources outside of the colony. For the carrier to carrier and carrier to affected crosses, dogs were bred that had no common ancestor for at least the two previous generations. Puppies were genotyped within several weeks of birth at the *TPP1* c.325 locus using an allelic discrimination assay that distinguishes the normal and mutant alleles (Awano et al., 2006). Dogs were housed and bred in AALAC-accredited facilities maintained by the University of Missouri Office of Animal Resources. Dogs were maintained on 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 U.S. 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.

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 in the affected dogs. Any dogs with evidence of vision compromise or ophthalmic conditions deemed threatening to vision were excluded from the study. All dogs received ophthalmic examinations monthly throughout the study. Examinations included visually-mediated behavioral assessment and slit lamp biomicroscopy (SL14; Kowa Co. Ltd., Tokyo, Japan), and indirect ophthalmoloscopy. Pupils were dilated with a short-acting mydriatic (tropicamide 1%; Alcon, Fort Worth, TX), and indirect ophthalmoscopy (12500, Welch Allyn Inc., Skaneateles Falls, NY, USA) was performed. Fundus photographs were taken following examination (Retcam Shuttle, Clarity Medical Systems, Pleasantown, CA) and archived electronically. Photos were reviewed to evaluate for abnormalities in retinal appearance.

2.2. Bone marrow harvest and mesenchymal stem cell culture

Bone marrow aspirates were collected from each dog at approximately 10–12 weeks of age using a modification of procedures described previously (Frimberger et al., 2006). Dogs were sedated with intramuscular injections of dexmedetomidine hydrochloride and morphine followed by induction of anesthesia with intravenous propofol administration to effect (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 during the aspiration process. A local intramuscular injection of lidocaine was administered just prior to aspirate collection.

Bone marrow was aseptically collected from the humerus. The hair on the forelimb over the proximal end of the humerus was clipped, shaved and surgically prepared. The proximal portion of the greater tubercle was palpated and a small incision over this structure was made using a #15 surgical blade. A 15 gauge Illinois sternal/iliac bone marrow needle was introduced through the incision and pushed into contact with the lateral portion of the greater tubercle in the fossa where the lateral glenohumeral ligament attaches. The needle was passed into the bone using a drilling motion aiming the needle at an angle to pass down the medullary cavity. When the needle had penetrated fully through the cortex, the needle was advanced down the medullary canal for a short distance and the stylet was removed. A 10 mL syringe containing 0.2 mL preservative-free heparin was attached to the needle, and approximately 5 mL of marrow was withdrawn while the needle was continuously rotated to prevent possible clot formation. The needle was then withdrawn and the marrow was expelled into 3 mL of sterile 1X Minimum Essential Medium (MEM) Alpha (Thermo Scientific cat. No. SH30265.02).

The bone marrow preparation was vortexed for 30 s and then spun for 10 min at 1591 × g (Unico Powerspin centrifuge, “Blood” preset) to pellet the cells. Using sterile procedures, the supernatant was removed and discarded and the cells were suspended in 5 mL of fresh stem cell culture medium (SCCM), consisting of 1X MEM alpha, 20% fetal bovine serum (Thermo Scientific cat. No. SH30088.03/Gibco 16000-077), and 1X penicillin/streptomycin (Thermo Scientific cat. No. SV30010). The cell suspensions were plated onto sterile 100 mm × 20 mm culture dishes and additional SCCM was added to each dish to bring the total volume to 10 mL. The cells were then placed in a sterile incubator at 37 °C with 5%