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Long-term enzyme replacement therapy improves neurocognitive functioning and hippocampal synaptic plasticity in immune-tolerant alpha-mannosidosis mice

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ABSTRACT

Alpha-mannosidosis is a glycoproteinosis caused by deficiency of lysosomal acid alpha-mannosidase (LAMAN), which markedly affects neurons of the central nervous system (CNS), and causes pathognomonic intellectual dysfunction in the clinical condition. Cognitive improvement consequently remains a major therapeutic objective in research on this devastating genetic error. Immune-tolerant LAMAN knockout mice were developed to evaluate the effects of enzyme replacement therapy (ERT) by prolonged administration of recombinant human enzyme. Biochemical evidence suggested that hippocampus may be one of the brain structures that benefits most from long-term ERT. In the present functional study, ERT was initiated in 2-month-old immune-tolerant alpha-mannosidosis mice and continued for 9 months. During the course of treatment, mice were trained in the Morris water maze task to assess spatial-cognitive performance, which was related to synaptic plasticity recordings and hippocampal histopathology. Long-term ERT reduced primary substrate storage and neuroinflammation in hippocampus, and improved spatial learning after mid-term (10 weeks+) and long-term (30 weeks+) treatment. Long-term treatment substantially improved the spatial-cognitive abilities of alpha-mannosidosis mice, whereas the effects of mid-term treatment were more modest. Detailed analyses of spatial memory and spatial-cognitive performance indicated that even prolonged ERT did not restore higher cognitive abilities to the level of healthy mice. However, it did demonstrate marked therapeutic effects that coincided with increased synaptic connectivity, reflected by improvements in hippocampal CA3-CA1 long-term potentiation (LTP), expression of postsynaptic marker PSD-95 as well as postsynaptic density morphology. These experiments indicate that long-term ERT may hold promise, not only for the somatic defects of alpha-mannosidosis, but also to alleviate cognitive impairments of the disorder.

1. Introduction

Alpha-mannosidosis is a lysosomal storage disorder (LSD) caused by deficiency of lysosomal alpha-mannosidase (LAMAN; coded by Man2b1). Loss of LAMAN activity results in intralysosomal accumulation of mannose-containing oligosaccharides in visceral cells, and in neurons of the peripheral (PNS) and central nervous system (CNS). The human phenotype is heterogeneous, but most frequently includes neurological symptoms such as hearing impairment and intellectual disability (Malm et al., 2014). Cognitive dysfunction in patients is correlated with elevated concentrations of oligosaccharides and neurodegeneration markers (e.g., Tau, GFAP) in their cerebrospinal fluid (Borgwardt et al., 2016). Unfortunately, therapeutic options for alpha-mannosidosis patients are limited, and retrospective analysis of bone marrow transplantation effects revealed variable outcomes of this invasive high-risk

Abbreviations: ACSF, artificial cerebrospinal fluid; BL, baseline; CNS, central nervous system; CON-MOCK, mock-treated control mice; CA, cornu ammonis; DG, dentate gyrus; ERT, enzyme replacement therapy; FAD, familial Alzheimer's disease; fEPSPs, field excitatory postsynaptic potentials; KO-MOCK, mock-treated knockout mice; KO-ERT, ERT-treated knockout mice; LAMAN, lysosomal alpha-mannosidase; LTP, long-term potentiation; LSD, lysosomal storage disorder; PB, phosphate buffer; PNS, peripheral nervous system; PS1, presenilin 1; REC, recording electrode; rh, recombinant human; SC, Schaffer collaterals; STIM, stimulating electrode; SVM, support vector machine; TBS, theta burst stimulation; TLC, thin layer chromatography; vGluT1, vesicular glutamate transporter 1.

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procedure (Mynarek et al., 2012). However, our continuously improving understanding of this and similar disorders inspired the development of therapeutic approaches that target different steps in the pathogenic cascade (Parenti et al., 2013). Contemporary variants of enzyme replacement therapy (ERT) are considered promising approaches, and have been clinically approved for several LSDs (Desnick and Schuchman, 2012). The development and application of murine (and other animal) models has been instrumental in this progress (Hemsley and Hopwood, 2010).

LAMAN-deficient mice, an established animal model for human alpha-mannosidosis (Stinchi et al., 1999), display a behavioral phenotype (which includes progressive motor and cognitive deficits) that mimics many aspects of the clinical condition (D'Hooge et al., 2005; Blanz et al., 2008; Caeyenberghs et al., 2006; Damme et al., 2011). This model has been used to test ERT approaches preclinically (Roces et al., 2004; Blanz et al., 2008). Highly dosed intravenous applied recombinant human (rh)LAMAN has been shown to cross the blood-brain barrier, and alleviate CNS storage in these mice. These studies inspired the first ERT study in patients (Borgwardt et al., 2013). However, the progressive immune response to the injected enzyme only allowed evaluation of short-term treatment regimens (<2 weeks), which failed to improve neurocognitive and other defects of higher brain function. To overcome these experimental limitations, immune-tolerant mouse models, which express inactive forms of the enzyme, have been generated for various LSDs (Matzner et al., 2007; Raben et al., 2003; Sly et al., 2001; Tomatsu et al., 2005).

In the present study, we used recently generated immune-tolerant LAMAN-deficient mice in the assessment of the functional effects of long-term ERT. Prolonged ERT already showed superior results to short-term treatment with respect to primary oligosaccharide storage and secondary neuroinflammation (Damme et al., 2015). Since hippocampus was amongst the best responding brain regions in this respect, we wanted to examine the therapeutic potential of long-term ERT on hippocampus-dependent higher cognitive functions, as well as synaptic plasticity, which is considered the cellular substrate for learning and memory abilities (Stuchlik, 2014). Targeting cognitive dysfunction is certainly clinically relevant, since intellectual improvement remains a major therapeutic objective in the clinical condition. We evaluated immune-tolerant LAMAN-deficient mice with specific focus on spatial-cognitive performance in the Morris water maze as the rodent equivalent of declarative memory abilities and complex learning in humans (Pooters et al., 2015). Treatment was initiated at 2 months of age, and the mice were evaluated in different water maze experiments, during the course of 9 months of weekly ERT treatment. Changes in learning and memory were linked to electrophysiological LTP recordings in hippocampal CA1 region, since hippocampal LTP was shown to be altered in LAMAN-deficient mice (D'Hooge et al., 2005). Spatial-cognitive assessment and hippocampal synaptic plasticity recordings were related to subregion-specific alterations in neuropathological and synaptic markers as well as ultrastructural analysis of CA1 excitatory synapses.

2. Material & methods

2.1. Animals and enzyme treatment

Immune-tolerant alpha-mannosidosis mice and control littermates (mixture of heterozygous and wild-type mice) were generated as described (Stinchi et al., 1999; Damme et al., 2015; hereafter referred to as alpha-mannosidosis and control mice). Mice were housed at standard laboratory conditions (12 h light/dark cycle, constant room temperature and humidity). Mice from different experimental groups were mixed housed. Behavioral testing took place during the light phase of the cycle. Food and water were available ad libitum.

ERT was initiated at an approximate age of 2 months and continued for 9 months, after which mice were sacrificed for electrophysiological, histological and/or biochemical analysis. rhLAMAN was derived from Chinese hamster ovary cells transfected with human α-mannosidase cDNA. Purification was performed as described previously (Borgwardt et al., 2013). Alpha-mannosidosis mice received weekly tail vein injections of rhLAMAN in a dose of 500 mU/g body weight (i.e., 15.6 mg/kg), or a comparable volume of saline solution. Consequently, three experimental groups were compared: CON-MOCK, KO-MOCK and KO-ERT (all n = 8, comprising 5 females and 3 males). Experimental protocols were approved by the ethical research committee of the KU Leuven or other relevant authorities, according to EC guidelines.

2.2. Determination of lysosomal enzymes

Preparation of detergent-containing tissue lysates, and determination of the specific activity of the lysosomal hydrolases β-glucuronidase, β-hexosaminidase, β-galactosidase, and α-mannosidase, was as described previously (Blanz et al., 2008), using a colorimetric assay and the following substrates: *p*-Nitrophenyl β-D-glucuronide *p*-Nitrophenyl-*N*-acetyl-β-D-glucosaminide, *p*-Nitrophenyl-β-D-galactopyranosid and *p*-nitrophenyl-a-D-mannopyranosid in substrate solution (0.1 M sodium citrate pH 4.6,0.08% NaN_3, 0.4% BSA, 0.15% NaCl). After incubation at 37 °C, the assays were stopped after addition of 0.4 M glycine/NaOH, pH 10.4. Absorbance was recorded at 405 nm (ε = 18,500 M^{-1} cm^{-1}).

2.3. Extraction of neutral oligosaccharides and thin layer chromatography (TLC)

Tissue samples (50 mg) were homogenized in 10 volumes (vol.) of dH_2O at 4 °C. After two thaw-freeze cycles followed by ultrasonification, proteins were precipitated and extracted by the subsequent addition of 4 vol. methanol and 4 vol. chloroform/H_2O (1:3). After desalting of the supernatant by incubation for 1 h at 4 °C with mixed-bed ion-exchange resin (AG 501-X8, 20–50 mesh, Bio-Rad) to remove charged molecules, the unbound material was lyophilized and resuspended in water. The aqueous oligosaccharide extracts were resuspended in HPLC-grade water and spotted onto Silica gel TLC plates (20 × 20 cm Silica gel F60; Merck, Darmstadt, Germany). Oligosaccharides were separated by n-butanol/acetic acid/water (100:50:50) development overnight followed by n-propanol/nitromethane/dH_2O (100:80:60) development for 4 h. After drying, plates were sprayed with 0.2% (weight/vol.) orcinol solution (20% H_2SO_4 dissolved in dH_2O) and heated at 110 °C until dark bands appeared.

2.4. Immunofluorescence microscopy of brain sections

Mice were perfused transcardially 24 h after the last injection with 0.1 M phosphate buffer (PB, pH 7.4) to wash out any remaining enzyme from circulation. After dissection, brains were postfixed in 4% paraformaldehyde for additional 4 h and subsequently transferred to 30% sucrose in PB overnight and finally sectioned on a Leica 9000 s sliding microtome (Wetzlar, Germany) into 50-μm-thick free-floating slices. After blocking unspecific antibody binding sites of the slices with blocking solution (0.5% Triton X-100, 4% normal goat serum in PB), the sections were incubated with the primary antibodies rat-anti-LAMP-1 (clone 1D4B), synaptic vesicle protein (clone SV2) (Developmental Studies Hybridoma Bank, Iowa City, USA), guinea-pig-anti-Synaptophysin and guinea-pig-anti-Synapsin (Synaptic Systems, Göttingen, Germany), Postsynaptic density protein 95 (PSD-95) (Abcam, Cambridge, UK), Calretinin (Swant (clone CD955) (Marly, Switzerland), Iba1 (Genetex, Alton Pkwy, CA) and rat-anti-CD68 (AbD Serotec, Edinburgh, UK), overnight at 4 °C in blocking solution. After 3 washing steps in washing buffer (0.25% Triton X-100 in PB), sections were incubated with AlexaFluor 488-conjugated secondary antibody for 2 h at room temperature (and AlexaFluor 594 for double-labelling), washed again 3 times in washing buffer, and finally coverslipped in