Evaluation of Neurodegeneration in a Mouse Model of INCL by MRI and Spectroscopic Analyses

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Introduction

The neuronal ceroid lipofuscinoses (NCLs), also known as Batten disease, are a group of common (1 in 12,500 births) heritable, progressive neurodegenerative lysosomal storage diseases of childhood. The infantile form, INCL, is the most devastating disease. Children with INCL are normal at birth but become blind by 2 years of age and brain dead by 4. They remain in this vegetative state for 6-8 years when death occurs. INCL is caused by inactivating mutations in the palmitoyl-protein thioesterase-1 (PPT1) gene. Many proteins are post-translationally modified by fatty acids such palmitic acid by forming thioester linkages on cysteine residues. While palmitoylation is essential for many proteins that require membrane anchorage for their function, deparmitoylation is equally important for their degradation and/or recycling. In this process PPT1 cleaves thioester linkages releasing palmitic acid from s-acylated proteins facilitating their degradation by lysosomal proteinases. Inactivation of PPT1 causes abnormal intracellular accumulation of s-acylated (palmitoylated) proteins leading to INCL pathogenesis. In this disease, the brain is severely affected due to the degeneration of cortical and hippocampal neurons, glial cell infiltration, and the presence of storage material called granular osmiophilic deposits (GRODS). However, the cerebellum and brain stem areas seem to remain relatively unaffected. The PPT1-knock-out (PPT1-KO) mouse has proven to be an excellent model to study the molecular mechanism of INCL, for these mice mimic virtually all clinical and pathological features of human INCL, including neurodegeneration and infiltration of the brain by glial cells. Since INCL diagnosis is often missed at early stages, the disease almost always progresses to an advanced stage. Thus, the pathologic progression of this disease is incompletely documented. The PPT-KO mouse model allows for early and continuous study of the progression of INCL and provides an opportunity to test novel therapeutic interventions. Here, we show that using magnetic resonance imaging (MRI) and spectroscopic analyses, changes in the brains of PPT1-KO mice can be detected as early as 4 months of age raising the possibility that these techniques may be used to evaluate the efficacy of novel therapeutic interventions in this animal model of INCL.

Materials and Methods

Age and sex matched PPT1-KO mice and their wild type (WT) littermates (n=4-5), at age 3, 4, 5 and 6 months were investigated. Mice were anesthetized with 1.5% isofluorane, a tail vein catheterized, and positioned in a steriotaxic holder. The body core temperature was maintained at 37^0C using a circulating water pad. MR imaging was performed on a horizontal 7T Bruker Avance scanner with the brain centered in a 72/25 mm transmit/receive coil ensemble. Fast spin echo, 1 mm multi slice axial images, specified via a tri-axial pilot scan, were acquired to encompass the whole brain (matrix 256x256, NA = 8, echos = 8, TR = 3000 ms and TE = 10 ms, FOV= 1.92 cm). An identical set of images with a gradient echo sequence was also acquired (TR/TE = 300/6 ms). Five T_2 (TE = 10 ms, 16 echos) and diffusion (TE= 30 ms, Δ = 20 ms, 4 b values 0-1500 mm/sec- in three orthogonal axes) weighted axial images, positioned approx. 6 mm posterior to the Bregma with 2 mm gaps towards the anterior, were acquired. Arterial spin labeled scans of four 1.5 mm thick slices (gap of 2 mm), origin at 6 mm posterior to the Bregma (labeling pulse length 2 ms with power of 81 mG/cm, matrix 64x64) were acquired. A localized ^{1}H spectrum of a 2.5 mm^3 voxel, positioned approx. 1 mm anterior to the Bregma, encompassing cortex, corpus collosum and CBF, were acquired with (TR/TE = 1500/16 ms, NA=512, 4k data points, SW=5kH, suppression pulse length = 12.5 ms) and without water (NA=4) suppression. The gradient echo sequence was repeated twice, after infusion of Gd^{2+} (0.2 cm^3 /kg body weight), and finally cerebral blood volume was measured by infusion of iron oxide (20 mg Fe/kg body weight). The brain volumes and areas under the MR spectral peaks were calculated using Bruker display software (Bruker Biospin Inc. Billerica, MA). T_2, Apparent diffusion coefficients (ADC), relative blood flow, cerebral blood volumes for selected anatomical areas of the cortex, corpus collosum, optic chiasm, thalamus were calculated using MATLAB (Mathworks Inc., Natick, MA).

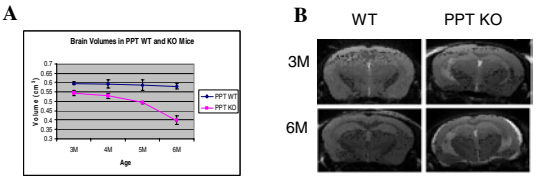

Figure 1: Changes in brain volume as shown by T_2 weighted images (a) and Gd^{2+} DPTA (b). The results in part A were calculated using a minimum of N=3.

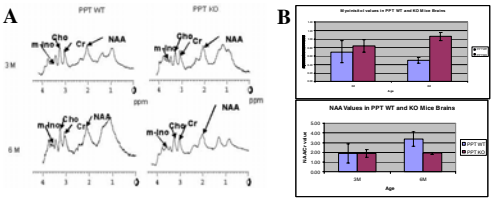

Figure 2: Spectrocopy results showing changes in brain composition. At 6 months, both the increase in myoinisitol and decrease in NAA values were significant. For all calculations, a minimum of two mice were used.

Results

Changes in total brain volume can be seen in the calculations from T_2 weighted images (**Figure 1A**) and the observation of Gd^{2+} in the brain indicated disruption of the Blood brain barrier in KO mice (**Figure 1B**). For the total brain volume, all P-values comparing WT and KO mice at each month are statistically significant ($p<0.05$). One consequence of the shrinkage of the brain is that increased fluid surrounding the brain is readily detected in KO mice but absent in WT mice. This change in PPT1-KO mice can be observed as early as 3-4 months of age. The spectroscopy data reveals the compositional changes that are occurring in the brain, which is seen in a quantitative analysis of the spectra of 3-month and 6-month old PPT1-KO and WT mice (**Figure 2A**). There is also evidence of decrease in neurons as demonstrated by both a decrease in NAA levels from 3 months to 6 months, and a corresponding increase in myoinisitol levels (**Figure 2B**). These differences were found to be significant in 6-month old mice, but not in those that are 3 months old, suggesting that these changes in the brain can first be observed between 3 to 6 months. Histology verifies that there is significant neuronal apoptosis in these animals. The T_2 measurements did not show significant difference between PPT1-KO and WT mice but the values of both groups decreased with age. Average ADC and Gd^{2+} intensity were higher in PPT1-KO mice at 5 and 6 months of age, but were not significantly different at 3 months. Increased cerebral blood volumes were found to be significant at 6 months of age in PPT1-KO mice, but not at an earlier age.

Discussion and Conclusions

There are readily detected MRI changes occurring in the brains of PPT1-KO mice that are characteristic of neurodegeneration. Changes in brain size (**Figure 1**) and composition (**Figure 2**) can be detected observed by volume calculations from T_2 weighted images and spectroscopy, respectively. Significant differences in brain volume are detected as early as 3 months in PPT1-KO mice. Increasing amounts of Gd^{2+} are observed around the cortex of the brain and in the corpus callosum starting at 3 months, and increasing over time in PPT1-KO mice. This suggests that the blood-brain barrier is disrupted in KO compared to WT mice. Changes in brain composition are apparent in the spectroscopic analyses of common neuronal markers. The neuron marker NAA decreases over time, confirming that there are less neurons present in the brain at 6 months than at 3 months. The increase in myoinositol levels indicates gliosis due to apoptosis of neurons. Both the qualitative and quantitative measures of brain volume, in combination with the changes in NAA and myoinositol levels can be measured in the brains of PPT1-KO mice beginning at 3 to 4 months of age. Our results suggest that a combination of MRI and spectroscopic analyses should be valuable in evaluating the efficacy of novel therapeutic approaches in this mouse model of INCL.