MRI/MRS of a Mouse Model for Canavan Disease

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Introduction

Canavan disease (CD) is an autosomal recessive leukodystrophy associated with spongy degeneration of the white matter of the brain, leading to mental retardation, megalencephaly and early death {1}. Brain histology in CD shows characteristic spongy degeneration of the white matter and astrocytic swelling, while neurons are spared. Aspartoacylase (ASPA) deficiency is the basic defect in CD. This enzyme hydrolyzes N-acetylaspartic acid (NAA) to aspartate and acetate. N-Acetylaspartic acid is abundantly synthesized in brain cells in human and other mammals. How a deficiency in the hydrolysis of NAA leads to spongy degeneration and the phenotypic characteristics of Canavan disease is not known.

Here, we characterize a mouse model for human Canavan disease generated by inactivating the murine ASPA gene by homologous recombination in embryonic stem cells. This model can be used for two main purposes: 1) to study the role of NAA in brain, and whether levels of this compound can be modulated. 2) to test gene therapy approaches, direct enzyme delivery and other therapeutic modalities so that a similar therapy could be developed for Canavan disease in children.

Methods

The isolation and characterization of the mouse ASPA targeting construct with truncation mutation will be described fully in a forthcoming paper.

All MRI/MRS experiments were performed using a 4.7 Tesla, 33-cm diameter bore system (Varian Inova, Palo Alto) equipped with a 10 gauss/cm (max.), 12.8 cm diameter gradient insert. A single-turn, 1.7 cm surface coil, tuned to 200 MHz was used to acquire MRI and MRS data. Mice were anesthetized with a 40 mg/kg dose of 2,2,2-tribromoethanol (avertin) for MRI/MRS studies. Proton MRI was performed using a gradient echo pulse sequence in order to set the voxel position for localized spectroscopy in the brain. The voxel was of the dimensions 4mm X 3mm X 3mm. The voxel was centered in the thalamus but included some brain stem and cortex as well. Spectra were acquired using the point resolved spectroscopy (PRESS) pulse sequence {2} Each of the two echo times was 40 ms, the repetition time was 2.5 sec. Each voxel was shimmed using the unsuppressed water peak in order to minimize spectral line widths. Subsequently, water suppression was achieved using three successive chemical shift selective 90-degree pulses (centered on the water peak) each pulse followed by a crusher gradient. Water suppressed spectra were acquired with between 160 and 400 signal averages, resulting in total spectral acquisition times of between 6.7 and 16.7 minutes. The resulting free induction decays were processed by Fourier transformation with a 5 Hz exponential apodization. Following baseline correction, spectral peak integrals for creatine/phosphocreatine (Cr, 3.05 ppm) and N-acetyl-aspartate (NAA, 2.02 ppm) were measured. Experimental groups for NMR spectroscopy included Canavan (+/+, n=6) Canavan heterozyogote (+/- n=4) and wild type (n=2) mice. Since Canavan heterozygote mice did not express Canavans disease, these mice were pooled with control mice. In this study the Cr peak served as the internal standard as previously reported .{3} Statistical comparison of the ratio of the NAA/Cr peak intensities was made using a t-test for differences between sham+control vs. Canavan expressors.

Multislice T2-weighted spin echo images were performed in two of the control mice and two of the Canavan expressing mice. Fourteen contiguous 1-mm slices were acquired with a TR of 3.0 sec, a TE of 50 ms, a field of view of 3cm X 3cm, 256 phase encode steps and 2 signal averages per step.

Results

Phenotypic characterization

The phenotype of the knock-out mouse was clearly different from the normal mouse. These mice were smaller and weighed 20-50% less than their litter mates.

MRI/MRS

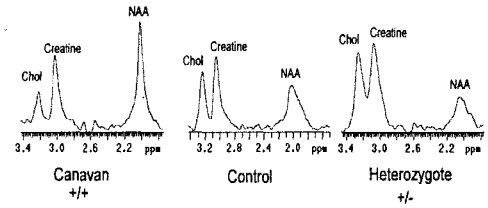
The NAA/Cr peak integral ratio was significantly higher (p=0.01) in Canavan expressing mice (2.28±0.42 mean/SEM) versus control mice (0.79 ±0.20). These results were corroborated by 400 MHz proton NMR spectra of perchloric acid extracts of Canavan and control mice.

T2-weighted images revealed areas of high signal intensity in the brain stem and diencephalon of Canavan-expressing mice, indicative of abnormally high water content in these regions. The area of highest image intensity appears to be centered in the midbrain.

Table: NAA/Cr in normal and homozygote knock-out mice for Canavan disease

Group	n	Mean NAA/Cr	SEM
Canavan (+/+)	6	2.28	0.42
Control (-/-,+/-)	6	0.79	0.20

P=0.003



Discussion

A knock-out mouse for Canavan disease has been created for the first time. The phenotype of the knock-out mouse, the MRI and MRS findings make the model as a useful tool for the study of the role of NAA and aspartoacylase in normal brain and maintenance of normal white matter. Such information would be important in understanding the pathophysiology of Canavan disease.

The MRI and MRS studies show the changes in the brain of these mice which are similar to the human disease. These findings maybe important in evaluating in vivo therapeutic interventions.

References

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