Decreased ADC in a Mouse Model of a Lysosomal Storage Disease

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Synopsis

Mice that lack the hexosamidase b gene (hexb-/-) are a model of the lysosomal storage disease, Sandhoff's disease. In this study, we utilize diffusion-weighted magnetic resonance imaging to evaluate physiological changes in the hexb-/- mouse brain in terms of fluid diffusivity as a consequence of GM2 ganglioside and glycolipid accumulation. The apparent diffusion coefficient (ADC) values in the cortex and striatum of the Hexb -/- mice and control mice at 3-4.5 months old were evaluated and verified with histology.

Introduction

The enzymatic hydrolysis of the GM2 ganglioside is catalyzed by the αβ-hexosaminidase (Hex) protein. There are two major isoforms of Hex: Hex A (composed of one α and one β subunit) and Hex B (composed of two β subunits). Tay-Sachs disease results from mutations of HEXA gene encoding the α subunit of Hex A. Sandhoff's disease, on the other hand, results from mutations of the HEXB gene encoding the β subunit(1). In both diseases, it has been reported that neurons are enlarged due to excess stored GM2 and related glycolipids in the lysosomal compartment (1).

Although the symptoms of both of this disease have been characterized, the precise mechanism of how GM2 specifically causes neurodegeneration is still not known. ***Quite interestingly, varying levels of GM2 accumulation have been reported in additional models of neurodegeneration***. Our goal was to determine if we could use DWI to assess *in vivo*, progressive neurodegeneration due to GM2 accumulation.

Methods

MRI: Diffusion-weighted imaging experiments were performed using a Bruker Avance Biospec, 9.4 T spectrometer, 21 cm bore horizontal imaging system (Bruker Biospin, Billerica, MA) with a 35 mm volume resonator. 6 Hexb -/- mice and 6 Hexb control mice (3-4.5 month old) were imaged with cardiac and respiratory combined gating (SA Instruments, Stony Brook, NY). During the imaging the animal body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY). One axial slice with 1.0mm thickness was obtained from each mouse brain. Images were acquired using a 4-shot EPI protocol with a diffusion gradient oriented along the slice direction, TR=1000ms, TE=35.8ms, δ=2.0ms, Δ=20.0ms, b=0, 300, 600 and 900 mm2/s, FOV=30mm, with a matrix size of 128 x 128, and 4 signal averages were acquired.

MRI Analysis: MRI images were analyzed using Image Sequence Analysis tool with Paravision software (Bruker Biospin, Billerica, MA) and code written in-house. 4 ROIs (region of interest) were selected at identical location for each dataset with 2 at the cortex and 2 at the striatum. The apparent diffusion values (ADC) were then calculated for each region.

Histology: After imaging, mice were perfusion fixed with 4% paraformaldehyde and brains were paraffin embedded and stained using a PAS staining kit (Sigma, St. Louis, USA).

Results

Our data demonstrates that on average there is a 12.9% decrease in the ADC values in cortex and a 17.0% decrease in striatum in Hexb -/- as compared to controls (**Figure 1**). There is statistically significant difference on ADC values between Hexb -/- and control in both cortex (### $p < 0.0017$) and striatum (*** $p < 0.0002$) based on a two-tailed Student's T-test, 95% confidence interval.

Figure 2a demonstrates a histological section from a control mouse. **Figure 2b** shows a representative histological section from a hexb-/- mouse. Note the preponderance of swollen lysosomes in the knockout (Fig 2b) as compared to the control (Fig 2a) (arrows point to examples). This lysosomal swelling is consistent with the decrease in the ADC we observed on the MRI images.

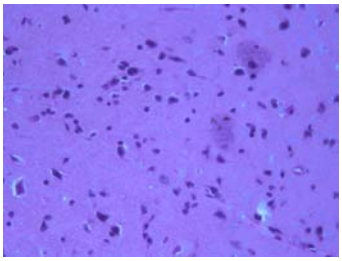

Figure 2a

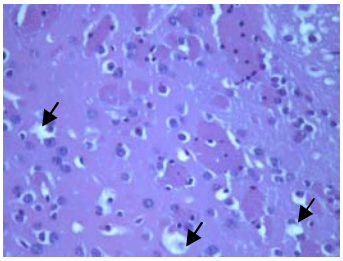

Figure 2b

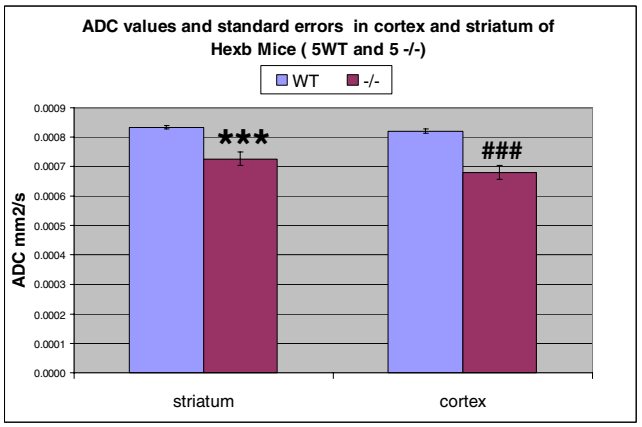

Figure 1

Discussion

The study demonstrates that the accumulation of GM2 ganglioside is detectable by diffusion-weighted MRI. Since gangliosides are particularly abundant in the nervous system and function in neuritic growth, cell recognition and neural transmission (1), diffusion-weighted MRI is an effective imaging method of ganglioside-related neuro-degenerative diseases compared to conventional T1 and T2 weighted imaging. Furthermore, this approach may provide additional directional diffusion information that is sensitive to ordered neuronal structures, which might provide a better understanding of the *in vivo* and longitudinal spatial distribution and accumulation of gangliosides in lysomsomal storage diseases as well as other forms of neurodegeneration.

References:

1. Phaneuf D, et al. Dramatically different phenotypes in mouse models of human Tay-Sachs and Sandhoff diseases. Hum Mol Genet. 1996 Jan;5(1):1-14.