Diffusion Tensor Imaging Measures Therapeutic Effects in a Mouse Model of Niemann-Pick Type C Disease at 23 Days of Age

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INTRODUCTION

Niemann-Pick Type C disease (NPC) is an irreversible neurodegenerative disorder without current treatment. It is thought to result from deficient intracellular cholesterol and/or ganglioside trafficking [1]. Although it is rare, NPC is particularly devastating because most NPC sufferers present in early childhood with progressive ataxia and neurodegeneration that leads to death in the second decade of life. A number of therapies are under development in animal models. Recently, a study has shown that treatment with single injections of the neurosteroid Allopregnanolone at day 7 in *Npc1*$^{-/-}$ mice significantly extended life span and delayed the onset of neurological symptoms [2]. However, the mechanism of action of this therapy is not known. This study reports results from DTI experiments carried out in mice at 23 days of age, just two days after weaning. At this early age, NPC mice demonstrate no symptoms of neurodegeneration, however DTI was able to detect deficiencies in myelin and normalization of these deficiencies with Allopregnanolone treatment.

METHODS

Twelve mice were studied: four control wild-type mice (WT), four untreated *Npc1*$^{-/-}$ mice (NPC), and four NPC mice treated with Allopregnanolone at day 7 (NPC-Allo). Allopregnanolone (Sigma/Aldridge) was dissolved in a 20% solution of beta-cyclodextrin in water at 1.25 mg/ml by brief sonication of the chilled solution and was injected subcutaneously at 25 mg/Kg. At weaning (about 21 days of age), tail tips were removed for genotyping. MRI was carried out on mice at 23 days of age using a Bruker Biospec 4.7 T instrument with 200 mT/m shielded gradients. Animals were anesthetized by isoflurane gas and placed into a custom holder which fits snuggly into a 20 mm Litz coil (Doty Scientific Inc). Body temperature was monitored with a fiber optic rectal probe and maintained at 37°C. DTI was carried out using a diffusion-weighted radial spin-echo sequence [3] with the following parameters: TR/TE=2s/54ms, matrix=128×128, FOV=1.92×1.92 cm^2, acquiring twelve contiguous 0.5 mm coronal sections. A total of seven images sets were collected, one without diffusion weighting and six with diffusion weighting (b=1065 s/mm^2, Δ/δ = 25/9 ms) along 6 non-colinear directions. The total scan time was 3 hours. After scanning, mice were perfused and their brain was removed for histopathology. Diffusion anisotropy parameters were calculated by standard algorithms on a pixel-by-pixel basis using programs written in IDL.

RESULTS and DISCUSSION

As seen qualitatively in Fig. 1A, there is a noticeable reduction of fractional anisotropy (FA) in the white matter tracks of NPC mice compared to WT littermate controls. Luxol fast blue stained sections of the three types of mice, Fig. 1B, show a reduction of myelin in the white matter of the NPC mouse when compared to the WT mouse. A normalization of myelin content in the NPC-Allo mouse towards that of the WT mouse is also apparent at this age. The results from a region of interest (ROI) analysis of the FA maps are shown in Fig. 2. There was a significant decrease in the FA of untreated NPC mice compared to WT mice in all the white matter ROIs. Furthermore, FA values in the NPC-Allo mice were higher than those of the untreated NPC also in all the ROIs. An analysis that is less prone to bias is a histogram analysis where the values of FA in the entire brain region are compared therefore a whole brain FA analysis of WT, NPC, and NPC-Allo mouse groups was also performed and results are summarized in Fig.3. The Volume Difference Index (VDI) is defined as the percentage of NPC (untreated or treated) mouse brain volume above a given FA threshold divided by the percentage of WT mouse brain volume above that same threshold. The dashed line (VDI=1) represents the case where brains have identical FA histograms and is included for reference. VDI decreases as FA threshold increases, indicating a preferential loss of brain with higher FA in NPC mice compared to WT mice. Additionally, there is a shift towards normal values in the treated NPC mice. For instance, compared to WT, NPC mice have only 21% of the brain volume with FA values above 0.7, whereas treated NPC mice have 43%.

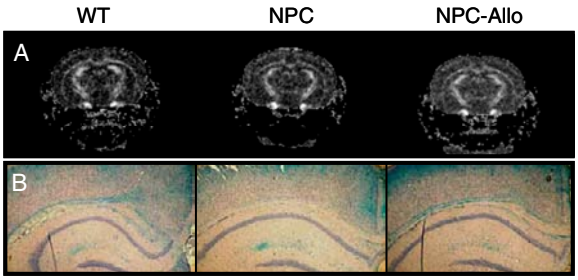

Fig 1. A) Representative FA maps of a WT mouse, an NPC mouse, and an NPC-Allo mouse. B) 4x magnification of the corpus callosum (CC) from brain sections stained with luxol fast blue (LFB) and Hematoxylin as a counter stain. LFB stains myelin in a turquoise color.

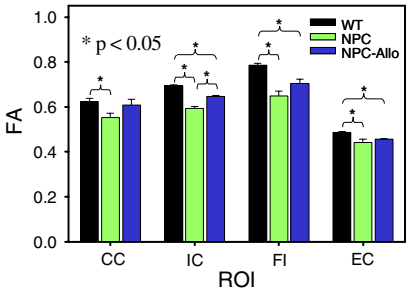

Fig 2. FA measured in ROIs from the WT, NPC and NPC-Allo mice. ROIs located in the corpus callosum (CC), internal capsule (IC), fimbria of the hippocampus (FI), and external capsule (EC).

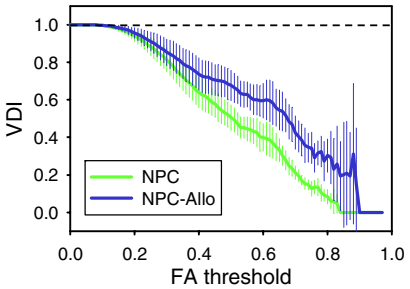

Fig 3. FA whole brain analysis of WT, NPC, and NPC-Allo plotting the volume difference index (VDI) versus FA. Standard error of the groups is indicated by vertical bars (n=4 each group).

CONCLUSIONS

This work represents the first demonstration of early, non-invasive detection of deficient myelination in the NPC mouse. The detection of deficient myelin at this early age in vivo using MRI is consistent with post mortem histological results and supports the idea that the NPC1 defect results in abnormal myelin formation [4] as opposed to secondary demyelination [5] in the NPC mouse. These results also suggest that one mechanism of action of allopregnanolone treatment is a restoration of myelin development.

References: [1] Patterson, *et al.*, in: Metabolic and Molecular Bases of Inherited Disease, pp.3611 (2001), [2] Griffin, *et al.*, Nat. Med. 10:704 (2004), [3] Trouard, *et al.*, *MRM*., 42:11 (1999), [4] Weintraub *et al.*, Acta Neuropathol. 74:374 (1978), [5] German *et al.* Neurosci 109:437 (2002).