Response to Therapy Measurement in a Mouse Model of Niemann-Pick Type C Disease via Diffusion Tensor Imaging

S. Lope-Piedrafita[1], C. M. Hicks[2], E. S. Chaitkin[3], I. Ahmad[3], R. J. Gillies[4,5], J-P. Galons[1], R. P. Erickson[3], T. P. Trouard[1,5]

[1]Radiology, University of Arizona, Tucson, AZ, United States, [2]Optical Sciences Center, University of Arizona, Tucson, AZ, United States, [3]Pediatrics, University of Arizona, Tucson, AZ, United States, [4]Arizona Cancer Center, University of Arizona, Tucson, AZ, United States, [5]Biomedical Engineering Program, University of Arizona, Tucson, AZ, United States

Introduction

Niemann Pick Type C (NPC) disease is a homozygous recessive disorder in the intracellular trafficking of low density lipoprotein derived cholesterol resulting in accumulation of intracellular unesterified cholesterol and sphingolipids [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. While there is currently no effective therapy for NPC disease, 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]. In both preclinical and clinical evaluation of therapies, it will be extremely useful to have a reliable, non-invasive means to track the progression of the disease and to monitor its response to therapy. In this effort, we have applied diffusion tensor imaging (DTI) to investigate the neurological abnormalities associated with NPC disease. This study reports results from DTI experiments carried out in control wild-type mice (WT), untreated *Npc1*$^{-/-}$ mice (NPC), and NPC mice treated with Allopregnanolone at day 7 (NPC-Allo7).

Methods

Twelve mice were studied: four WT, four NPC, and four NPC-Allo7 mice. 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 65-69 days of age (end-stage for untreated NPC mice) using a Bruker Biospec 4.7T instrument with 200 mT/m shielded gradients. Animals were anesthetized by isoflurane gas and placed into a custom holder which fit snuggly into a 20 mm Litz coil (Doty Scientific Inc). Body temperature was monitored with a fiber optic rectal probe and maintained at 37^{o}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. Diffusion anisotropy parameters were calculated by standard algorithms on a pixel-by-pixel basis using programs written in IDL.

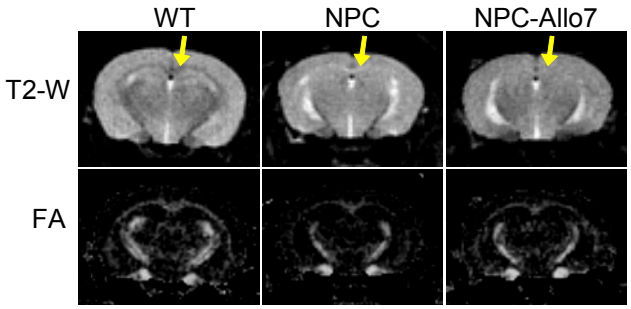

Fig 1. Representative T2-weighted images and FA maps of a wildtype mouse (WT), an untreated *Npc1*$^{-/-}$ mouse (NPC) and an *Npc1*$^{-/-}$ mouse treated with Allopregnanolone at day 7 (NPC-Allo7).

Results and Discussion

Fig.1 shows representative T2-weighted images and fractional anisotropy (FA) maps of a WT mouse, an NPC mouse and an NPC-Allo7 mouse. Arrows in T2-weighted images indicate the approximate location of the corpus callosum which appears hypointense, hyperintense, and isointense, respectively, when compared to the surrounding tissue. Highly mylenated structures have low T2 and appear dark in T2-weighted images as seen in the WT brain. This same region is hyperintense in the NPC mice and is most likely a sign of diminished myelin content. NPC-Allo7 mice showed some level of normalization towards WT mice. FA maps reveal a generally reduced anisotropy in the white matter regions of the untreated NPC brain as compared to the wild type. Differences are particularly apparent in the corpus callosum and within the internal capsule and external capsule. Day-7 treated NPC mouse brain also shows decreased anisotropy in white matter but does not appear as severe as in the untreated NPC mouse.

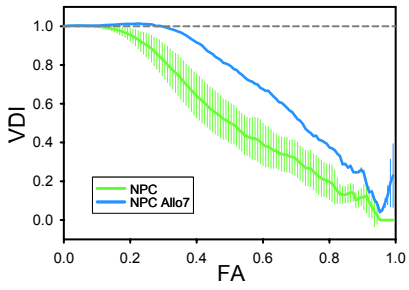

Fig 2. FA whole brain analysis of WT, NPC, and NPC-Allo7 plotting the volume difference index (VDI) versus FA. VDI is defined as the percentage of NPC (untreated or treated) mouse brain volume above a given FA threshold divided by the percentage of WT mouse brain volume above that same threshold. The dashed line (VDI=1) represents the case where brains have identical FA histograms and is included for reference. Standard error of the groups is indicated by vertical bars (n=4 each group).

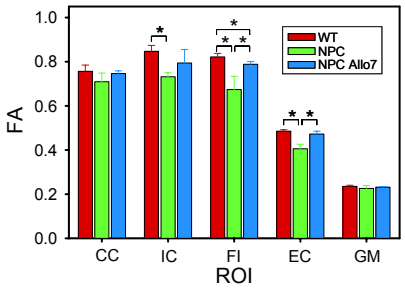

Fig 3. FA measured in regions of interest (ROIs) from the WT, NPC and NPC-Allo7 mice. ROIs located in the corpus callosum (CC), internal capsule (IC), fimbria of the hippocampus (FI), external capsule (EC), and cortical gray matter (GM). (* $p < 0.05$).

Whole brain FA analysis of WT, NPC, and NPC-Allo7 mouse groups are shown in Fig.2. The VDI (see Fig. legend) decreases as FA threshold increases, indicating a preferential loss of brain with higher FA in NPC mice compared to WT mice. However, for the treated NPC mice the reduction is not as severe as for their untreated counterparts. For example, compared to WT, NPC mice have only 20% of the brain volume with FA values above 0.8, whereas treated NPC mice have 38%. Notice that the variance among NPC-Allo7 mice is very small compared to untreated mice.

Fig. 3 shows the region of interest (ROI) analyses carried out on the FA maps. There was a significant decrease in the FA of untreated NPC mice compared to WT mice in the IC, FI and EC. FA values in the NPC-Allo7 mice were higher than those of the untreated NPC for all white matter ROIs.

In conclusion, we have found that DTI was able to measure a decrease in diffusion anisotropy in white matter in an NPC mouse model compared to WT mice. Furthermore, DTI was able to detect the effect of a therapeutic agent known to improve neurological symptoms in this mouse model.

References [1] Patterson, *et al*., in: Metabolic and Molecular Bases of Inherited Disease, pp.3611 (2001), [2] Griffin, *et al*., Nature Medicine 10:704 (2004), [3] Trouard, *et al*., *MRM*., 42:11 (1999).