Partial preservation of white matter in a murine model of Niemann-Pick type C disease with therapeutic intervention: An ex vivo DTI study

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Introduction. Niemann-Pick type C (NPC) disease is an inherited metabolic disorder resulting from a defect in either the *NPC1* or *NPC2* gene. Loss of functional NPC1 or NPC2 protein leads to widespread intracellular accumulation of unesterified cholesterol and glycosphingolipids (GSLs). Effects on the central nervous system are particularly deleterious and include a relentless decline in motor coordination and intellectual function, eventually culminating in an untimely death. We have shown amelioration of disease in a mouse model of NPC1 by administration of either miglustat (Mig) or 2-hydroxypropyl-β-cyclodextrin (CD). Treatment of NPC1$^{-/-}$ mice with Mig or CD alone slows clinical progression and ameliorates pathological manifestations of NPC disease (1, 2). We also have evidence suggesting a synergistic effect when both drugs are used as a combination therapy. A critical, unanswered question is whether either treatment, or a combination of both treatments, effectively reduces neuronal cell death and neuroaxonal dystrophy. DTI studies of NPC have suggested reduced fractional anisotropy and increased mean diffusivity (3, 4). Thus, we used ex vivo DTI in a mouse model of NPC1 disease to determine if white matter has been preserved by monotherapy or both drugs.

Methods. Animals: NPC1$^{-/-}$ mice, along with WT littermates, were treated with Mig (NPC1: n = 4, 12-18 wks; WT: n = 9, 12-30 wks), CD (NPC1: n = 9, 10-30 wks; WT: n = 9, 10-30 wks) or combination of Mig and CD (Com. NPC1: n = 8, 12-30 wks; WT: n = 6, 12-30 wks) as previously described (2). Additionally, NPC1$^{-/-}$ (n = 6, 12 wks) and WT (n = 13, 10-30 wks) mice were treated with saline as a control group. Mice were sacrificed at specified time points by transcardial perfusion with 0.9% saline solution followed by 4% paraformaldehyde in PBS. Whole brains were immersion fixed in 4% paraformaldehyde/PBS overnight and then stored in PBS at 4°C until DTI acquisition. MR Data Acquisition: The samples were immersed in fomblin and scanned using a 9.4 T Varian Direct Drive animal MRI/MRS system (Agilent Technologies, Inc. Santa Clara, CA). A 14-mm ID single loop receive coil (Doty Scientific, Inc., Columbia, SC) and a 7-cm ID ^{1}H transmit coil (m2m Imaging Co., Cleveland, OH) were used for data acquisition. A fast spin echo sequence was employed to obtain 30 coronal T2-wt images (78 × 78 × 500 μm^3, no gap) with the following parameters: TR/TE = 5000/44 ms, echo train length = 4, NEX = 8. DTI images were acquired using a multi-(8) shot spin-echo, navigator corrected echo-planar imaging sequence in 30 coronal slices of 500 μm thickness (no gap) with in-plane resolution of 156 × 156 mm^2, TR/TE = 6000/39 ms, flip angle of 90°. The non-diffusion-weighted (b0) and 30 directions images (Jones 30 gradient directions, b-value 916 s/mm^2) were acquired with δ = 3.2 ms and Δ = 9.8 ms. Data Analysis: Diffusion tensor fitting was performed using MIPAV (medical imaging processing and visualization, NIH, Bethesda, MD). Region of interests (ROIs) of corpus callosum (CC) and fimbria (FI) were manually outlined on the fractional anisotropy (FA) maps at all slices that contained the structure of interest. Then the ROIs were applied to eigenvalue maps to extract axial diffusivity (AD) and radial diffusivity (RD). The data are presented as mean ± SD. For group comparisons, Student's *t*-test was performed with $p < 0.05$ considered significant.

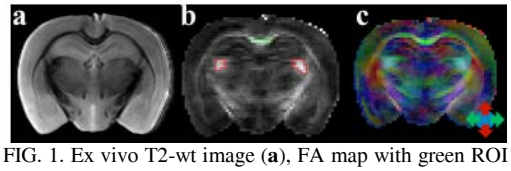

FIG. 1. Ex vivo T2-wt image (**a**), FA map with green ROI of CC and red ROI of FI (**b**) and DEC map (**c**) of a fixed mouse brain.

FIG. 2. FA maps of fixed brains from NPC control (a), Mig-treated NPC mouse (b), CD-treated NPC mouse (c) and WT mouse (d).

Results. Figure 1 shows the T2-wt image, FA and direction-encoded color (DEC) maps of a fixed mouse brain as well as ROIs outlined in CC and FI. FA maps in Figure 2 show increased FAs in white matter in NPC1$^{-/-}$ mice treated with Mig (Fig.2b), CD (Fig.2c), combination of two drugs (not shown) compared to NPC1$^{-/-}$ control mice (Fig.2a). With treatment, FA in corpus callosum increased from 0.49 ± 0.03 (NPC-control) to 0.53 ± 0.01 (NPC-Mig), 0.63 ± 0.06 (NPC-CD), and 0.63 ± 0.03 (NPC-Com), respectively, although these values remained lower than WT controls (0.69 ± 0.06). Similar results were also observed in fimbria region: 0.48 ± 0.03 (NPC-control), 0.55 ± 0.03 (NPC-Mig), 0.64 ± 0.03 (NPC-CD), 0.63 ± 0.03 (NPC-Com) and 0.70 ± 0.05 (WT-control), as shown in Fig.3a. FA of NPC1$^{-/-}$ mice treated with Mig is lower than that with CD ($p < 0.001$) or combination ($p < 0.01$). The combination treatment yielded FA values in white matter are comparable to CD alone. Treatment of WT mice with Mig, CD, or combination did not alter FA in either brain region assessed (data not shown). The improvement of FAs in NPC1$^{-/-}$ mice treated with different drugs was mainly a result of reduced radial diffusivity, not axial diffusivity, as shown in Figure 3. There was no significant difference of axial diffusivity among any groups of NPC1$^{-/-}$ mice (Fig.3b). Radial diffusivity in white matter decreased significantly with CD or combination treatment, but not with Mig treatment. (Fig.3c).

Discussion and Conclusion. Both miglustat and 2-hydroxypropyl-β-cyclodextrin have shown a remarkable effect on reducing Purkinje cell death and delaying onset of motor deficits in a mouse model of NPC1 disease (1, 2). An important, unanswered question is whether either treatment, or a combination of both treatments can effectively prevent white matter deterioration in NPC disease. The neurological symptoms of NPC typically appear after a period of normal early development and reflect progressive degeneration of widespread brain regions. White matter degeneration has been suggested to occur as early as 9 days of age (5). Our data show that starting early postnatal treatment with miglustat or cyclodextrin can partially delay the degeneration and preserve some white matter structure. White matter FA improved significantly in NPC1$^{-/-}$ mice treated with CD or combination of CD and miglustat. Radial diffusivity was reduced by about 30% with CD or combination treatment, which indicates CD can partially preserve myelination or prevent dysmyelination during brain growth in NPC1$^{-/-}$ mice. In conclusion, we found that treatment with CD alone or in combination with miglustat led to significant preservation of white matter myelin in treated NPC1$^{-/-}$ mice. Cyclodextrin was more effective than miglustat in preserving myelin, which is consistent with the greater disease amelioration provided by cyclodextrin in preserving neurological function and prolonging survival in the NPC1 mouse model.

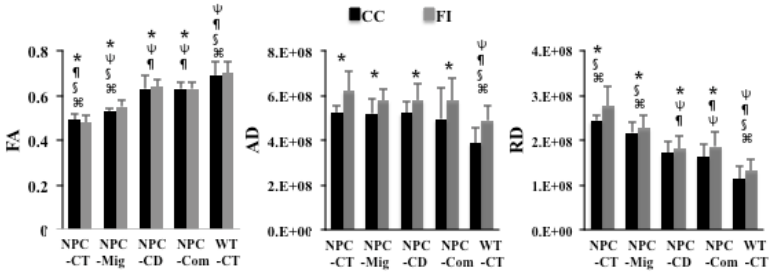

FIG. 3. Results of fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD) of NPC control (NPC-CT), NPC treated with Mig (NPC-Mig), with CD (NPC-CD), with combination of Mig and CD (NPC-Com), and WT control (WT-CT). Data from different ages were pooled together. Black bar: corpus callosum (CC); grey bar: fimbria (FI). Error bars are SD. $p < 0.05$: *vs. WT-CT; $^{\Psi}$vs. NPC-CT; ¶vs. NPC-Mig; §vs. NPC-CD, $^{\Box}$vs. NPC-Com.

References. 1. Zervas M, et al. Curr Biol. 2001; 11:1283-1287. **2**. Davidson CD, et al. PloS ONE. 2009; 4 :e6951. **3**. Walterfang M, et al. Neurology. 2010; 75: 49-56. **4**. Totenhagen JW, et al. J Magn Reson Imaging. 2012; 35: 528-536. **5**. Ong WY, et al. Exp Brain Res 2001; 141: 218-231.