

Gene therapy evaluated using in vivo diffusion tensor imaging

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Introduction

Diffusion tensor imaging (DTI) is one of the most commonly used imaging modalities to assess central nervous system (CNS) tissue integrity. It has been widely applied for diagnosis and monitoring therapy efficacy. However, its use to monitor gene therapy of CNS diseases has not been common. Globoid-cell Leukodystrophy (GLD) is an inherited demyelinating disease caused by a deficiency of the lysosomal enzyme Galactosylceramidase (GALC). A previous study demonstrated dramatic synergy between CNS-directed AAV2/5 gene therapy and myeloreductive bone marrow transplantation (BMT) in the murine model of GLD (twitcher). In the present study, in vivo DTI was employed to evaluate the effect of gene therapy of twitcher mice demonstrating the treatment induced recovery of white matter integrity.

Materials and Methods

Animals Heterozygous twitcher (GALC^{+/-}) mice on a congenic C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME). The homozygous twitcher mice (GALC^{-/-}) were obtained by heterozygous by heterozygous matings. The genotype was determined by twitcher-specific PCR. Four groups of animals were examined: untreated wildtype (wt), untreated twitcher (twitcher), AAV2/5 only twitcher (AAV), and combination treated twitcher (AAV+BMT).

Treatment Regimens The treatment of AAV+BMT group consisted of AAV2/5 injections on day 2 or 3 of life and BMT on the following day consisting of an intrathecal injection and 6 intracranial injections. Intrathecal injections were performed on the neonatal spine, in the midline at the upper lumbar vertebral column. A total of 15 μ l of virus was injected using a 50 μ l Hamilton syringe. For intracranial delivery, 2 μ l of AAV2/5 was injected at both hemispheres: a) forebrain: 2mm lateral and 1mm caudal to bregma and 1.5mm deep, b) thalamus: 2mm lateral and 2.5mm caudal to bregma and 2.5mm deep; and c) cerebellum: 1mm lateral and 3mm posterior to lambda and 2.5mm deep. For BMT, the bone marrow donors were sex-matched syngenic WT (GALC^{+/+}) mice expressing GFP under the CAGGS promoter which were maintained as a separate colony. Newborn mice received 400 rads of total body γ radiation from Cs¹³⁷ source for conditioning followed by injection of 10⁶ GFP(+) sex-matched unfractionated bone marrow cells in 100 μ l volume in to the superficial temporal vein.

DTI Five 36 days old mice from each group were employed for in vivo DTI examination on a 4.7 T magnet utilizing respiratory gated spin-echo diffusion-weighted sequence with actively decoupled volume (6-cm inner diameter, RF excitation) and surface coil (16 mm x 9 mm, signal receiver). All images were obtained with acquisition parameters of TR 1.2 sec (gated acquisition), TE 38 ms, Δ 18 ms, δ 7 ms, slice thickness (1.0 mm), zero filled spatial resolution (38 μ m x 38 μ m), total data acquisition time ~ 1.0 hr, (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), and (1,0,-1), and b = 0 and 1.0 ms/ μ m².

Results and Discussion

Decreased $\lambda_{||}$ and increased λ_{\perp} was apparent in the in vivo DTI parameters maps (Fig. 1 a – h). In DWM (i and k), the $\lambda_{||}$ of AAV+BMT was statistically insignificantly lower than that of the control suggesting a positive treatment effect. In the AAV only treatment the $\lambda_{||}$ was statistically significantly lower than that of the control suggesting a less effective axonal preservation. A trend of steadily decreased λ_{\perp} was seen from the untreated mutant to the wild type control suggesting the more effective myelin preservation in AAV+BMT treatment while AAV was also showed some positive myelin preservation. In VLWM (j and l), both AAV+BMT and AAV treated groups showed statistically significantly improved $\lambda_{||}$ and λ_{\perp} .

Although the $\lambda_{||}$ and λ_{\perp} were not completely back to the control value after AAV+BMT or AAV treatments, the significantly improved axon and myelin preservation suggests the efficacy of these treatments. The current finding supports that in vivo DTI may be used to monitor gene therapy in leukodystrophy and potentially other white matter diseases.

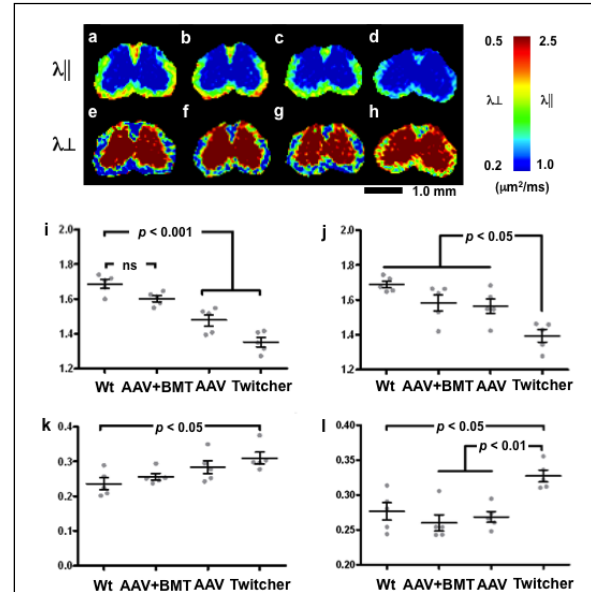


Figure 1. The representative in vivo DTI derived $\lambda_{||}$ (a - d), λ_{\perp} (e - h) maps of wt (a, e), AAV+BMT (b, f), AAV (c, g), and untreated (d, h) mice showing close to normal $\lambda_{||}$ and λ_{\perp} after treatments. The ROI analysis quantitatively compared $\lambda_{||}$ of dorsal (i, DWM) and ventral-lateral (j, VLWM) white matter among all group, showing a better efficacy of AAV+BMT at the axonal preservation of DWM. Both treatments preserved axonal integrity toward the wt status. Similar improvement in λ_{\perp} was also evident in DWM (k) and VLWM (l) where remyelination was equally significant in VLWM for both treatments while DWM again showed slightly better improvement in AAV+BMT treatment.