Evaluation of cerebellum and globus palidus by in vivo diffusion tensor imaging in a rat model of bilirubin encephalopathy
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Target audience: Neuroscientist, radiologist, neurologists, MR scientists with interest in preclinical models.

Objective: Bilirubin encephalopathy can either present in the newborn as Kernicterus, a devastating disorder of the globus pallidus resulting in dystonic cerebral palsy, or as a chronic encephalopathy preferentially involving the cerebellum in patients with Crigler-Najjar syndrome, a genetic disorder due to recessive mutations in UDP-glucuronosyltransferase 1A (UDPG1A). While the UDPG1A mutant Gunn rat has served as a model of bilirubin encephalopathy, no imaging evaluation of this model has been reported. The objective of this study was to compare in vivo diffusion tensor imaging (DTI) studies to the severity of neurological disability and neuropathology, in order to determine whether this imaging method can serve as a biomarker for therapeutic studies and further enhance our current understanding of bilirubin encephalopathy and secondary dystonia.

Methods: Jaundiced Gunn rats were either treated with saline (jj-C, n=3) or with 80mg/kg intraperitoneal Sulfadimethoxine (jj-S, n=3) at postnatal day 15 (P15) and compared to wild type controls (WT, n=3). Sulfadimethoxine displaces unconjugated bilirubin and leads to acute severe dystonia. A Bruker horizontal 11.7T scanner was used to acquire high-resolution T2-weighted and diffusion tensor images in vivo at P17. Experiments were carried out with a 72 mm diameter quadrature transmit volume coil and a 4-channel phase array receive coil. Data were acquired using a 3D diffusion weighted gradient and spin echo sequence with the following parameters: TE/TR = 27.5/600 ms, 6 diffusion directions, b = 1000 s/mm² and a resolution of 0.125 x 0.125 x 0.4 mm³. Total scan time was 1.5 hours. Region of Interest analysis was performed in middle cerebellar peduncle (MCP) and the inferior cerebellar peduncle (ICP), and the globus pallidus (GP) in consecutive slices in Fractional Anisotropy (FA) maps and direction-encoded color maps. In addition, cerebellar volumes were measured via manual segmentation.

Results: All animals in the jj-S group developed ataxia with profound dystonia, whereas animals in the jj-C group exhibited ataxia without dystonia. Cerebellar volume analysis showed highly significant decreases in jj-C and jj-S animals compared to WT controls, whereas no significant difference in cerebellum volume was found between jj-C and jj-S animals (Fig.1b). The MCP showed significant decreases in FA in all jaundiced animals (jj-C and jj-S) compared to WT (Fig.2), whereas the ICP solemnly indicated a trend towards lower FA in jaundiced animals. Interestingly, we found a significant decrease of FA in jj-S compared to WT and jj-C in the GP-region (Fig.3b), whereas FA in this region did not differ between WT and jj-C animals.

Discussion: While this is a pilot study, our data implicates two interesting findings. First, all jaundiced Gunn rats exhibit white matter changes in the MCP in addition to profound cerebellar atrophy, which is reported in patients with Crigler-Najjar syndrome. Interestingly, an acute change in bilirubin toxicity induced by Sulfadimethoxine did not have any significant effect on cerebellum or cerebellar tracts. A second finding is that acutely dystonic Gunn rat showed an FA decrease in the GP, an area that shows bilirubin accumulation pathologically and is found to be abnormal in newborns with Kernicterus. DTI may therefore serve as an in vivo correlate for acute bilirubin toxicity in the dystonic Gunn rat.

Fig.1: A) T2-weighted cerebellar cross section B) Cerebellar volume measured via manual segmentation (***≤ 0.0001)
Fig.2: Mean FA comparison of MCP (*≤ 0.05; **≤ 0.01)
Fig.3: A) GP-region of interest outlined in FA-map B) Mean FA comparison of GP (*≤ 0.05), C) postmortem section illustrating the yellow bilirubin staining in GP