MRI and histological evidence for the blockade of Cuprizone-induced Demyelination in C57/Bl6 mice by Quetiapine

P. Chandran[1], J. Upadhyay[1], S. Markosyan[2], A. Lisowski[3], W. Buck[3], G. B. Fox[1], M. Day[1], and F. Luo[1]

[1]Translational Imaging and Biochemistry, Abbott Laboratories, Abbott Park, Illinois, United States, [2]Neuroscience Discovery, Abbott Laboratories, Abbott Park, Illinois, United States, [3]Cellular and Molecular Exploratory Toxicology, Abbott Laboratories, Abbott Park, Illinois, United States

Introduction

Schizophrenia is a severe psychiatric disorder with a largely unknown etiology. A disruption in white matter connectivity, specifically oligodendrocytic loss, has been implicated in the etiogenesis of schizophrenia. Non-invasive *in vivo* high spatial and temporal resolution MRI techniques have become important tools in the study of pathogenesis and pathophysiology of schizophrenia. Recently, there has been growing number of pre-clincial studies using cuprizone to induce oligodendrocytic loss that mimics the white matter abnormalities associated with schizophrenia [1]. Histological and in vivo imaging assessments have demonstrated that the administration of cuprizone can reliably induce demyelination in brain regions implicated in the pathophysiology of schizophrenia such as the corpus callosum, hippocampus and the cortex [2] [3] [4]. Furthermore, cuprizone-induced behavioral deficits have been shown to be reversed by first and second generation antipsychotics [5] [6] [7]. The cuprizone induced white matter demyelination mouse model may afford an opportunity to image the integrity of white matter pathways, such as the corpus callosum using structural and DTI MRI. There are two aims of the current study. The first aim is to evaluate the impact of sub-chronic cuprizone exposure on the integrity of the corpus callosum using two in vivo MR imaging techniques: T2-weighted and DTI. The second aim is to verify whether quetiapine attenuates cuprizone-induced demyelination in vivo using the same techniques.

Materials and Methods

Animals: 20 female mice of the C57BL/6NTac background were utilized in this study. The animals were divided into 4 treatment groups (n = 5/group). The first treatment group (control) was fed a regular chow diet and given distilled water for 6 weeks. The second treatment group (QTP) was fed regular chow diet and given QTP mixed with distilled water to drink for 6 weeks. The third treatment group (CPZ) was fed regular chow for 1 week followed by CPZ containing chow for remaining 5 weeks. Similar to the control group, this group of mice was given distilled water for 6 weeks. The last treatment group (CPZ+QTP) was fed regular chow for 1 week followed by CPZ containing chow for remaining 5 weeks. QTP was mixed in distilled water for administration to this group for the 6 week study duration. *MRI experiments:* T2 and DTI were conducted in a 7.0T /21 cm horizontal magnet housed with a 20 G/cm magnetic field gradient insert. Following anesthesia and before putting the mouse in the scanner for conducting the imaging experiment, each mouse was placed inside a dual-coil small animal restrainer. T2-weighted anatomical images were acquired using the RARE pulse sequence with TR = 2000 ms, effective TE = 100 ms, matrix = 256 x 256, FOV = 2.56 cm x 2.56 cm, inter-plane resolution = 100 µm x 100 µm, nine 1.0-mm slices, and four averages. The parameters for DTI are as follows: TE = 20.6 ms, TR = 3200 ms, bandwidth = 300 kHz, and one signal average with phase cycling was used. The field of view and matrix size were 25.6 mm × 25.6 mm and 128 × 128 and the inter-plane resolution was 200 × 200 µm; slice thickness = 1000 µm. Two non-diffusion-weighted (b0) images and 30 diffusion weighted directions (b value 750 s/mm2) were acquired with δ = 3 ms and Δ = 10 ms. The total imaging time was 14 min 56 s. For the quantification of diffusion anisotropy, fractional anisotropy (FA) was used. *Histological Analysis:* Histological (LFB) and immunohistological (MBP) assessments were conducted on brain tissue harvested from these animals. *Data Analysis:* All MRI data analysis were performed using the AFNI and FSL software packages. Region of interest (ROI) over corpus callosum (CC) were manually outlined based on a mouse brain atlas. Similarly, regional mean FA values of corpus callosum were obtained from the calculated FA maps. For quantifying the T2 findings, an inverse ratio of mean T2 signal intensity of ROI (corpus callosum) to a reference region (lateral ventricle, Vnt) was used to quantify signal enhancement or reduction.

Results

Myelin breakdown in cuprizone-exposed mice as visualized by in vivo T2 and DTI mapping is confirmed by the reduction of myelin basic protein in the corpus callosum by immunohistochemistry (MBP) method as well as histological (LFB) staining. However, pathological changes in white matter were either prevented or alleviated in cuprizone-exposed mice co-administered with quetiapine.

Fig.1: T2 (Fig. A) and DTI (Fig. B) maps for different treatment conditions.

Fig.2: LFB (Figs. A&B) and MBP (Figs. C&D) optical density (OD) findings for different treatment conditions
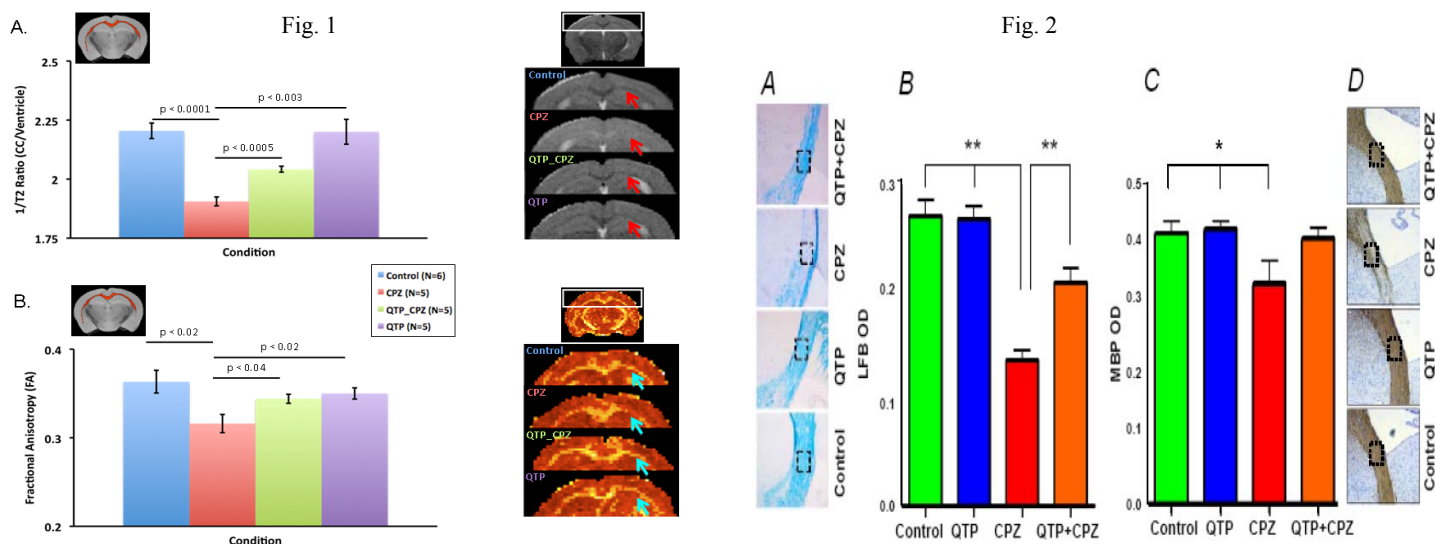
Discussion

These results suggest that the cuprizone-exposed C57BL/6 mouse is a potential animal model to investigate the impact of treatments on white matter abnormalities in schizophrenia. The current study is the first report using in vivo imaging to demonstrate (1) the deleterious effects of sub-chronic CPZ exposure on the integrity of the corpus callosum; (2) that quetiapine, when co-administered with cuprizone, blocked the copper chelator induced demyelination; (3) demyelination in the corpus callosum was confirmed by histological staining (LFB) and immunohistochemical (IHC) staining (MBP). The current study further demonstrates the ability of T2-weighted and DTI MR imaging techniques in detecting drug induced changes in white matter. These results demonstrate the utility of these MRI techniques in the drug discovery and development process. These techniques could aid in establishing a translatable biomarker and help in develop a better understanding of the schizophrenia disease process.

References. [1] Xu, H., *et al.* Behav Neurosci, 123 (2009) 418-29. [2] Wu, Q.Z., *et al.* J Magn Reson Imaging, 27 (2008) 446-53. [3] Xu, H., *et al.* Behav Neurosci, 123 (2009) 418-29. [4] Yang, H.J., *et al.* Brain Res, 1270 (2009) 121-30. [5] Xiao, L., *et al.* Mol Psychiatry, 13 (2008) 697-708. [6] Xu, H., *et al.* Front Behav Neurosci, 4 8. [7] Zhang, Y., *et al.* Schizophr Res, 106 (2008) 182-91.