NeuroImage xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Prenatal alcohol exposure reduces magnetic susceptibility contrast and anisotropy in the white matter of mouse brains☆

Wei Cao [a,b,1], Wei Li [a,1], Hui Han [a], Shonagh K. O'Leary-Moore [c], Kathleen K. Sulik [c], G. Allan Johnson [d], Chunlei Liu [a,e,*]

[a] Brain Imaging and Analysis Center, Duke University, Durham, NC, United States
[b] Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[c] Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC, United States
[d] Center for In Vivo Microscopy, Duke University, Durham, NC, United States
[e] Department of Radiology, Duke University, Durham, NC, United States

ARTICLE INFO

Article history:
Accepted 21 August 2014
Available online xxxx

Keywords:
Quantitative susceptibility mapping
Magnetic susceptibility anisotropy
FASD
DTI
White matter

ABSTRACT

Prenatal alcohol exposure can result in long-term cognitive and behavioral deficits. Fetal alcohol spectrum disorder (FASD) refers to a range of permanent birth defects caused by prenatal alcohol exposure, and is the most common neurodevelopmental disorder in the US. Studies by autopsy and conventional structural MRI indicate that the midline structures of the brain are particularly vulnerable to prenatal alcohol exposure. Diffusion tensor imaging (DTI) has shown that abnormalities in brain white matter especially the corpus callosum are very common in FASD. Quantitative susceptibility mapping (QSM) is a novel technique that measures tissue's magnetic property. Such magnetic property is affected by tissue microstructure and molecular composition including that of myelin in the white matter. In this work, we studied three major white matter fiber bundles of a mouse model of FASD and compared it to control mice using both QSM and DTI. QSM revealed clear and significant abnormalities in anterior commissure, corpus callosum, and hippocampal commissure, which were likely due to reduced myelination. Our data also suggested that QSM may be even more sensitive than DTI for examining changes due to prenatal alcohol exposure. Although this is a preclinical study, the technique of QSM is readily translatable to human brain.

Introduction

Fetal alcohol spectrum disorder (FASD) is an umbrella term used to describe the range of structural and functional defects that result from prenatal alcohol exposure. Included are long-term cognitive and behavioral abnormalities such as deficits in memory and attention (Streissguth et al., 1994), impairments in language ability (Steinhausen et al., 1982), fine motor dysfunction (Kyllerman et al., 1985), executive function deficits (Connor et al., 2000; Noland et al., 2003), and low intelligence (Alati et al., 2008). It is estimated that FASD occurs in as many as 2–5% of young school children in the US and Western Europe (May et al., 2007). Although FASD is a very common developmental disorder, our understanding of the associated brain abnormalities present within this spectrum remains incomplete.

Clinical and basic research has shown that prenatal alcohol exposure can affect multiple aspects of brain development including neurogenesis, gliogenesis, and myelination (Miller, 1988, 1993; Phillips, 1989). Conventional structural MRI has revealed both brain dysmorphology and volumetric changes (Lebel et al., 2011), with abnormalities involving median brain structures being frequently noted. In fetal alcohol syndrome (FAS), the syndrome that is at the severe end of the FASD spectrum, the corpus callosum is commonly affected (Bookstein et al., 2002; Swayze et al., 1997). Employing DTI to quantify abnormalities in the white matter fiber tracts of prenatal alcohol-exposed human brains, Ma et al. (2005) reported lower fractional anisotropy and higher mean diffusivity (MD) in the genu and splenium of the corpus callosum than in controls. A number of more recent DTI studies have confirmed the vulnerability of the corpus callosum to prenatal alcohol-exposure-induced damage (Lebel et al., 2008; Li et al., 2009; Sowell et al., 2008; Wozniak et al., 2006, 2009). In addition, DTI analyses employing tract-based spatial statistics (TBSS) have found reduced FA values in other white matter regions including the cingulate (Sowell et al., 2008) and superior-frontal

☆ Sponsor: NIH grant P41EB015897, R01MH096979, U01-AA017124, U01-AA021651, and P60-AA011605 and National Multiple Sclerosis Society grant RG4723.

* Corresponding author at: Brain Imaging and Analysis Center, Duke University School of Medicine, 2424 Erwin Road, Suite 501, Campus Box 2737, Durham, NC 27705, United States. Fax: +1 919 681 7033.
E-mail address: chunlei.liu@duke.edu (C. Liu).

[1] Wei Cao and Wei Li contributed equally to this work.

http://dx.doi.org/10.1016/j.neuroimage.2014.08.035
1053-8119/

tracts connecting the occipital lobe with inferior frontal and parietal lobes (Fryer et al., 2009). The reduced FA and increased radial diffusivity (RD) (Li et al., 2009) have largely been attributed to reduced myelination.

Myelin may also be studied based on the magnetic susceptibility property of white matter quantitatively and at high spatial resolution. It has been suggested that susceptibility may be even more sensitive and specific to myelination in the white matter than diffusion metrics. Liu et al. (2011) reported that loss of myelin sheath around axons in a transgenic dysmyelinating shiverer mice led to a near complete loss of phase and susceptibility contrasts between gray and white matter while FA and radial diffusivity were reduced by less than 20%. These results suggest that myelin is the predominant source of susceptibility difference between deep gray and white matter. In a recent study, Lodygensky et al. (2012) evaluated phase contrast changes during early development of mouse brains. They showed that phase contrast correlated with myelin content assessed by histology, while the gray-white matter phase contrast remains unchanged after iron extraction. Lee et al. (2012) also showed that frequency contrast is substantially reduced in mice with significant myelin loss induced by a cuprizone diet. In addition, magnetic susceptibility anisotropy of the white matter is thought to directly reflect the contents of myelin lipids (Li et al., 2012). Together, these studies indicated the potential value of magnetic susceptibility for imaging myelin.

In this study we quantitatively evaluated magnetic susceptibility of white matter in a mouse model of prenatal alcohol exposure (Godin et al., 2010). This mice model mimics human prenatal alcohol exposure occurred at the middle through the end of the third week of brain development, when the fetus brain undergoes its final growth spurt. Automatic ROI-based analysis was employed to assess quantitative susceptibility values of major midline fiber tracts in FASD and control groups using DTI and QSM, respectively. Furthermore, susceptibility anisotropy of major white matter fiber bundles was also evaluated and compared between the two groups. Volume changes of corresponding major white tracts were also measured. The study demonstrated that QSM can detect abnormalities in brain white matter in this FASD model and suggest that it may be even more sensitive than DTI.

Methods

Animal model

All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of at the University of North Carolina at Chapel Hill and Duke University. The procedures employed for prenatal ethanol exposure were the same as described by Godin et al. (2010). Briefly, female C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME) were housed in a temperature and humidity-controlled AAALAC-approved environment. Standard laboratory chow and water were available ad libitum. For breeding, 1–2 females were placed with one male for 2 hours. Detection of a copulation plug was defined as gestation day 0 (GD 0). On the beginning of GD 7, pregnant dams were randomly assigned to either an ethanol or control group, weighed, and administered either an intraperitoneal (ip) dose of 25% ethanol (2.9 g/kg) or an equivalent dose of Ringer's solution. Four hours later a second ethanol or Ringer's solution dose of equal volume and concentration was administered to each of the dams in the respective groups. This ethanol administration paradigm has previously been employed, yielding a mean peak blood ethanol concentration (BEC) of 440 mg/dl. This ethanol concentration is high enough to induce a range of CNS abnormalities without substantially increasing resorption with the objective of identifying even the most severe consequence of ethanol's dysmorphogenic effects (Godin et al., 2010). Following ethanol or Ringer's solution administration, dams were left undisturbed until birth.

The first day after birth was denoted as postnatal day 1 (PN1). All the litters were maintained in a central animal care facility with a 12-hour light/dark cycle and offered free access to water and food. On PN45, two group pups (7 with prenatal alcohol exposure and 7 with prenatal Ringer's solution exposure) were selected randomly as the ethanol group and the control group, respectively.

Magnetic resonance imaging

Brains were perfused using a transcardial access with a 1:10 mixture of ProHance-buffered formalin. Specimens were immersed in buffered formalin for 24 hours and then moved to a 1:200 solution of ProHance/salin to shorten T1 and reduce scan time. Specimens were scanned at 9.4 T (Oxford 8.9-cm vertical bore; GE 12.5X EXCITE console) using a 3D spoiled-gradient-recalled (SPGR) sequence. The scan parameters were as follows: matrix size = 512 × 256 × 256, FOV = 22 × 11 × 11 mm^3, flip angle = 90°, TE = 4.432 ms, TR = 50 ms, and scan time = 54.6 minutes. Diffusion tensor images (DTI) were acquired using a diffusion-weighted 3D spin-echo sequence (Jiang and Johnson, 2010) with the same FOV and matrix size. The other parameters were as follows: TE = 12 ms and TR = 100 ms. One image volume was acquired without diffusion weighting. Six diffusion-encoding directions were used at a *b*-value of 1500 s/mm^2 to allow the calculation of diffusion tensor. The encoding directions were (1 0 1), (1 0–1), (1 1 0), (1–1 0), (0 1 1) and (0, 1–1). Total scan time for DTI was 12 hours and 45 minutes.

Data analyses

Taking into consideration the limited sample size ($n = 7$ per group), all images were down-sampled to 60 μm isotropic spatial resolution from the native 43 μm resolution to ensure sufficient SNR (representing a 65% increase in the SNR of magnitude images). The diffusion-weighted images (DWI) from all directions were averaged to obtain an isotropic diffusion weighted image and used to extract the brain tissue by thresholding. Although the non-diffusion weighted image can also be used for brain extraction, we found that the isotropic weighted image was more convenient as it suppressed fluid and tissues outside the brain. All phase and susceptibility analysis were conducted in STI Suite (Duke University) (Li et al., 2014). Specifically, the phase of the SPGR data was unwrapped using a Laplacian-based phase unwrapping method (Li et al., 2011). The background phase was removed using the V-SHARP method, using the DWI-determined mask as an input (Li et al., 2011, 2014; Wu et al., 2012). Magnetic susceptibility was then obtained from the local tissue phase by solving an inverse problem using the LSQR method (Li et al., 2011). DTI parameters including FA values, MD values and eigenvalues, eigenvectors were computed as previously defined (Basser and Pierpaoli, 1996). All computations were conducted in Matlab R2010a (MathWorks, Natick, MA).

The calculated quantitative susceptibility maps and DTI-derived parameters were then analyzed as outlined in Fig. 1. Specifically, FMRIB's nonlinear image registration tool (FNIRT) (Oxford University, UK) was used to spatially register the native images to a standard-space template in the Waxholm Space (Johnson et al., 2010) (step 1). The transformation matrix was optimized based on the registration performed on the magnitude images of the gradient echo data. Masks for regions of interest (ROI) in selected white matter fiber bundles were automatically segmented using a previously defined brain atlas (Ali et al., 2005; Badea et al., 2007). These ROI masks included anterior commissure, corpus callosum, and hippocampal commissure. Reverse transformation into each subject's native space was carried out using invwarp (step 2). The extracted ROIs were also transformed back to the original images including susceptibility images and DTI images (step 3). The accuracy of the transformation was visually inspected for each map using the ITK-SNAP software (Yushkevich et al., 2006). ROIs that clearly exceeded the tissue boundary were revised accordingly (only one mouse in the

Please cite this article as: Cao, W., et al., Prenatal alcohol exposure reduces magnetic susceptibility contrast and anisotropy in the white matter of mouse brains, NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.08.035