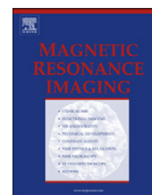
Magnetic Resonance Imaging 33 (2015) 551–558

Contents lists available at ScienceDirect

Magnetic Resonance Imaging

journal homepage: www.mrijournal.com



Bilateral common carotid artery occlusion induced brain lesions in rats: A longitudinal diffusion tensor imaging study

Xuxia Wang, Fuchun Lin, Yunling Gao, Hao Lei *

State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Center for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, P. R. China

ARTICLE INFO

Article history:
Received 30 October 2014
Revised 14 February 2015
Accepted 15 February 2015

Keywords:
Diffusion tensor imaging
Axial diffusivity
Radial diffusivity
Demyelination
Axon damage

ABSTRACT

Bilateral common carotid artery occlusion (BCCAO) has been widely used to reproduce the white matter (WM) and gray matter (GM) damage associated with chronic cerebral hypoperfusion (CCH). This study investigated whether diffusion tensor imaging (DTI) could be used at the early stages of disease to assess brain damage induced by BCCAO. To this end, DTI, together with histological methods, was used to evaluate the progression of WM lesions and GM neurodegeneration following BCCAO. The DTI was sufficiently sensitive to detect WM abnormalities in selected regions of the brain at 4 weeks after BCCAO. These abnormalities may indicate damage to the myelin and axons in the optic nerve (ON) and optic tract (OT). Our longitudinal results showed that DTI could be used to detect abnormalities of the WM and GM in select regions of the brain as early as 2 days after ligation. The DTI parameter patterns of change were region-specific throughout the detection time course. Lesions of the external capsule (EC) and periventricular hypothalamic nucleus (Pe) have not been thoroughly studied before. We found that the EC and Pe were both vulnerable to BCCAO and that the associated lesions could be detected using DTI. The current study demonstrated that in vivo DTI could potentially be used to measure WM damage evolution in a BCCAO rat model as well as early brain injury following CCH.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Chronic cerebral hypoperfusion (CCH) is an important factor in brain damage associated with aging and vascular dementia [1–5]. For example, white matter (WM) lesions often accompany CCH in the aging brain [6]. A rat model with permanent bilateral common carotid artery occlusion (BCCAO) has been proposed to reproduce the effects of CCH. In the rat brain, BCCAO usually leads to WM lesions and gray matter (GM) neurodegeneration. Previous studies [7,8] showed that the lesions included myelin rarefaction and vacuolization, axon damage, microglial activation, and astrogliosis in the optic nerve (ON) and optic tract (OT). These lesions were long-lasting. The GM hippocampus, neocortex, and superior colliculus suffered delayed neuronal loss [9], transient gliosis [10] and long-lasting gliosis [11], respectively, after BCCAO. Thus, there is a need for improved, noninvasive, and highly sensitive imaging methods to be developed for the early detection of brain damage as well as its evolution following BCCAO in vivo. These techniques would be especially useful if they could be combined with histopathological methods. They could also be used in pathological assessments of imaging indices in aging as well as vascular dementia studies.

A recent study by Sorial et al. [12] showed that magnetic resonance imaging (MRI) tools such as diffusion tensor imaging (DTI) could be used to detect the diffusion of subtle WM and GM abnormalities at 7 weeks after BCCAO. This indicated that DTI could potentially be used to longitudinally track the development of CCH-induced brain damage. A dynamic DTI evaluation of a BCCAO rat model is important not only in understanding the course of CCH-induced brain lesions, but also in developing imaging biomarkers in the evaluation of new neuroprotective drugs. Up to now, few studies have used the DTI technique to longitudinally assess rat brain damage induced by BCCAO.

In the present study, we measured longitudinal DTI index changes in a rat model of BCCAO. The measurements were performed at 2 days, 1 week, 2 weeks, and 4 weeks after BCCAO. Histological evaluations were performed at 4 weeks after BCCAO to verify DTI findings. The primary objective was to determine whether DTI measurements were sufficiently sensitive to detect abnormalities in the rat brain in the early stages following BCCAO and to determine whether DTI could provide useful information regarding the nature and time course of CCH-induced brain lesions.

* Corresponding author at: Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, West No.30 Xiao Hong Shan, Wuhan 430071, P. R. China. Tel.: +86 27 8719 8542; fax: +86 27 8719 9291.
E-mail address: leihao@wipm.ac.cn (H. Lei).

http://dx.doi.org/10.1016/j.mri.2015.02.010
0730-725X/© 2015 Elsevier Inc. All rights reserved.

2. Material and methods

2.1. Animal preparation and surgical procedures

The animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All of the animal protocols were approved by the institutional animal care committee of the Wuhan Institute of Physics and Mathematics. A total of 16 male Wistar rats, approximately 8 weeks of age and weighing 200–230 g, were used. The rats were housed in a room with an ambient temperature of 23 ± 1 °C and a 12-h light–dark cycle. They had free access to food and water. Each rat was anesthetized with an intraperitoneal injection of 5% choral hydrate solution (6 ml/kg body weight). A median incision was made in their necks. Their bilateral common carotid arteries were separated and isolated. For the rats assigned to the BCCAO group (BCCAO, n = 9), the bilateral carotid arteries were double-ligated with 4-0 sutures. For the rats in the control group (CON, n = 7), the bilateral carotid arteries were manipulated as in the BCCAO group, but without being ligated. The wounds were then closed. The body temperature of the rats was maintained (with a heating lamp) at 37 ± 1 °C during surgery as well as during recovery from anesthesia.

2.2. Magnetic resonance imaging measurements

MRI measurements were performed at 2 days, 1 week, 2 weeks, and 4 weeks after ligation on a 7 T/20 cm Bruker Biospec scanner. A volume coil was used for radiofrequency pulse transmission and a quadrature surface coil was used for signal detection. The rats were anesthetized by isoflurane in pure O_2 (3% for induction, 1.5%–2% for maintenance) delivered via a nose cone during the imaging sessions with their respiration monitored continuously.

DTI was performed with a spin-echo 4-shot echo-planar imaging (EPI) sequence, an encoding scheme of 30 gradient directions homogenously distributed on the unit sphere, and the following acquisition parameters: TR 5000 ms, TE 26 ms, FOV 3 cm × 3 cm, slice thickness of 0.8 mm, matrix size of 128 × 128, Δ 14 ms, δ 3 ms, two b values (0 and 800 s/mm^2) and 4 averages.

2.3. Histological evaluations

After the MRI examination at 4 weeks, the anesthetized rats were perfused with 300 ml saline and subsequently 300 ml 4% paraformaldehyde dissolved in 100 mmol/l phosphate-buffered saline (PBS, pH = 7.4). Their brains were removed, post-fixed in the same fixative for at least 24 h, and stored in a refrigerator at 4 °C until examined.

The brains were sectioned on a cryostat microtome (Leica, Germany). Coronal brain sections were obtained with a thickness of 15 μm or 30 μm. Slices at Bregma 2.52 mm (including the ON) were sectioned continuously for Luxol fast blue (LFB) staining, immunocytochemical glial fibrillary acidic protein (GFAP) staining, and hyperphosphorylated neurofilament (SMI-31) staining respectively. Slices at Bregma 1.44 mm (including the external capsules (EC), striatum (CPu), and motor and sensory cortexs (CTX)) were sectioned continuously for LFB staining, Nissl staining, immunocytochemical GFAP staining, and SMI-31 staining respectively. Slices at Bregma −2.4 mm (including the OT and periventricular hypothalamic nucleus (Pe)) were sectioned continuously for LFB staining, Nissl staining, immunocytochemical GFAP staining, and SMI-31 staining respectively.

For LFB staining, 15 μm-thick brain sections were incubated in a 0.1% LFB solution overnight at 60 °C. After reaching room temperature, these sections were first rinsed with 95% ethanol and then washed in distilled water. Differentiation was completed by briefly rinsing them in 0.05% lithium carbonate and 70% ethanol and then returning them to water until the contrast between the white and gray matter was maximized. The LFB-stained sections were then counterstained with toluidine blue.

For Nissl staining, 15 μm-thick brain tissue sections were immersed in a 0.1% toluidine blue solution for approximately 1 min, washed with distilled water, and then rinsed in 80% ethanol, 95% ethanol, 100% ethanol,100% ethanol, and 100% xylene subsequently for 5 min followed by mounting.

For immunohistochemistry, antigen retrieval was performed with 30 μm-thick brain sections. After cooling to room temperature, the endogenous peroxidases were inactivated via immersion in 3% H_2O_2 for 15 min, 3 washes in PBS, and incubation with 0.2% Triton X-100 for 30 min at 37 °C. After 3 rinses with PBS, the slides were blocked with 10% normal goat serum for 1 h to block nonspecific antibody interactions. Then, the diluted primary antibodies (GFAP, 1:100 PBS, and SMI-31, 1:500 PBS) were incubated for 2 h at 37 °C. After washing, the GFAP immunolabeling was detected with Histostain SP kit from Zymed with diaminobenzidine as the substrate (except for the ON); for the ON, the GFAP immunolabeling was detected with fluorescein isothiocyanate-conjugated goat anti-rabbit; the SMI-31 immunolabeling was detected with a secondary antibody (Alexa Fluor 488 goat anti-mouse).

2.4. DTI data analysis

Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) maps were acquired using the toolbox provided by the Bruker PARAVISON 5.0 software. Using the PARAVASION 5.0 software, the Regions of Interest (ROIs), representing different anatomical structures, were traced manually on the FA (i.e., without interpolation) maps for each rat with guidance from the Paxinos digital atlas [13]. Then, the ROIs were shifted to the identical position on the MD, AD and RD maps, and the values of DTI

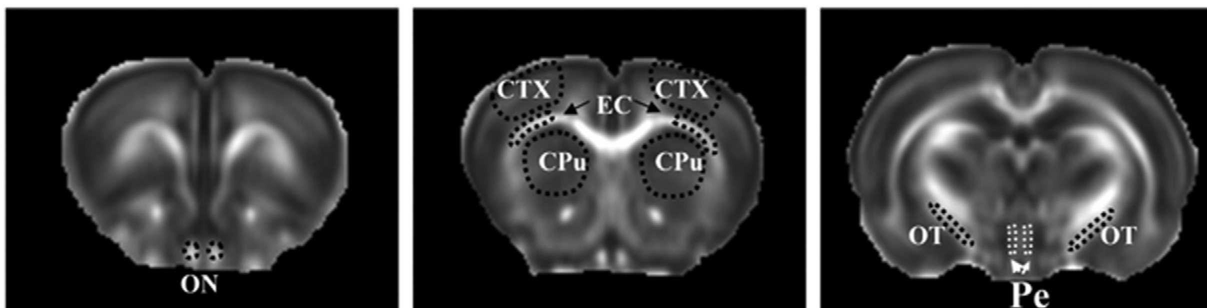


Fig. 1. The ROIs are manually drawn from the FA maps of the rat brain. ON: optic nerve, CTX: motor and sensory cortex, EC: external capsule, CPu: striatum, OT: optic tract, Pe: periventricular hypothalamic nucleus.

Download English Version:

https://daneshyari.com/en/article/1806277

Download Persian Version:

https://daneshyari.com/article/1806277

Daneshyari.com