Formalin fixation alters water diffusion magnitude but not anisotropy in infarcted brain

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Introduction

Magnetic resonance diffusion tensor imaging (DTI) is a widely used tool for the non-invasive investigation of tissue pathology and morphology. It has been suggested that DTI parameters characterize intrinsic features of tissue microstructure. Numerous studies have reported DTI examination of fixed tissues (1-4). Previously, we demonstrated that the anisotropy of water diffusion in formalin-fixed normal mouse brain is the same as that observed *in vivo* (5). Notably, DTI data can be obtained on *post mortem* tissue with extremely high spatial resolution as compared with *in vivo* studies since a long acquisition time is possible for fixed tissue. The question remains whether anisotropy remains unchanged in fixed, injured brain. The purpose of this study is to compare the DTI parameters of infarcted mouse brain *in vivo* and *ex vivo*.

Materials and Methods

Animal preparation

Five male mice (C57BL/6) weighing 20 – 30 g were subjected to permanent MCAO (6). DTI was conducted on each mouse 2.5 hours after surgery. Mice were anesthetized using isoflurane/O_2 (7% for induction and 2% for maintenance). Core temperature was maintained at 36.5°C using circulating warm water. After the appropriate anesthesia plane was reached, mice were placed in a custom-made, magnetic-resonance-compatible restraining device to immobilize the head. A 1.5 cm outer diameter circular surface coil was placed on top of the head to serve as the receiver for the MR signal. At the conclusion of MR examination, mice were deeply anesthetized and perfused through the left cardiac ventricle with phosphate buffered saline (PBS) followed by 10% formalin in PBS.

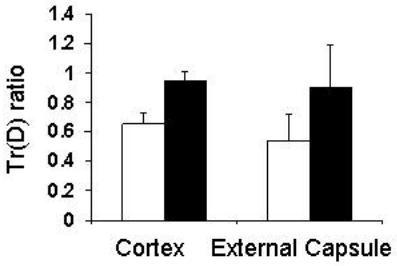

Fig. 1

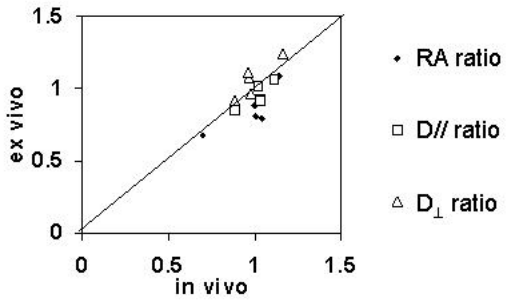

Fig. 2

Diffusion Tensor Imaging

A conventional multi-slice spin-echo imaging sequence modified by adding the Stejskal-Tanner diffusion sensitizing gradient pair was employed for acquisition of the required series of diffusion weighted images (DWI). The *in vivo* diffusion weighted images were acquired with TR = 1.5 s, TE = 50 ms, Δ = 25 msec, δ = 10 msec, slth = 0.5 mm, FOV = 1.5 cm, data matrix 128×128 (zero filled to 256× 256). Diffusion sensitizing gradients were applied along six directions: [Gx,Gy,Gz] = [1,1,0], [1,0,1], [0,1,1], [-1,1,0], [0,-1,1], and [1,0,-1]. Two diffusion sensitizing factors or b-values (0 and 0.768 ms/μm^2) were used for acquisition of the DWI series. The same acquisition parameters were employed for *ex vivo* measurements.

Data Analysis

Four quantitative indices including the trace of the diffusion tensor (Tr(D) = $\lambda_1 + \lambda_2 + \lambda_3$), relative anisotropy (RA), trace-normalized axial diffusivity ($D_{\|} = \lambda_1$ / Tr(D)), and trace-normalized radial diffusivity ($D_{\perp} = (\lambda_2 + \lambda_3)$ / (2×Tr(D))) were measured in the cortex and external capsule. The ratios of each index were calculated by dividing the values of the ipsilateral side by the values of the controlateral side.

Results

Acute ischemia caused a 35% and 46% decrease in Tr(D) in the ipsilateral cortex and external capsule respectively (Fig. 1). However, Tr(D) was the same ipsilateral and contralateral to the stroke after formalin fixation. However, diffusion anisotropy, as measured by RA, $D_{\|}$, and $D_{\perp}$, exhibits close agreement between *in vivo* and *ex vivo* measurements in both cortex and external capsule (Fig. 2). The correlation coefficients for *in vivo and ex vivo* RA, $D_{\|}$, and $D_{\perp}$ are 0.83, 0.82 and 0.87, respectively.

Discussions

The current data show that the Tr(D) decrease associated with stroke *in vivo* is not preserved after tissue fixation. Thus, the inherent difference in water diffusion caused by tissue fixation is likely to mask the decreased diffusion coefficient caused by ischemia *in vivo*. The preservation of diffusion anisotropy suggests that its use in fixed tissues is justified, even in the presence of ischemic injury.

Reference

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