Longitudinal changes of diffusion tensor imaging in acute stages of post-mortem animal brain tissue decomposition

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1 Introduction

The three-dimensional displacement of water molecules, as measured with diffusion tensor imaging (DTI), provides information on tissue micro-architecture in a non-invasive manner. As such, DTI has been used extensively for the study of tissue integrity (particularly white matter) in several neurological disorders and normal neurodevelopment and aging. Furthermore, DTI has been utilized to provide evidence of brain tissue decomposition following death [1-3]. However, in most previous studies, brain tissue was generally kept at a low temperature (4 °C) and/or processed for histology, thus altering the normal course of tissue decomposition. The goal of our study was to follow the diffusion characteristics of brain tissue following death, with the animal's corpse in the most natural conditions maintaining the brain inside the skull and at room temperature.

2 Methods

Three male adult California rabbits were included in this study. All animal procedures were approved by the Institutional Review Board. The rabbits were sedated with intra-muscular xylazine prior to anesthesia using intra-venous penthobarbital. The anesthetized animals were scanned, then euthanized within the scanner using an overdose of penthobarbital. Scanning continued at 2-hour intervals for a total of 10-14 hours, during which the animals' core temperature was monitored using a rectal thermometer. All imaging was performed using a 3T General Electric Discovery MR750 scanner and a 32-element human head coil. The spin-echo echo-planar DTI sequence consisted of 7 non-diffusion weighted volumes (b=0 s/mm^2) and 99 directionally sensitized diffusion weighted volumes (b=750 s/mm^2). Each volume consisted of 19 slices with voxel dimensions of 0.89×0.89×1.5 mm^3 (TR/TE=6000/94.3 ms). Scanning was repeated twice to improve signal-to-noise ratio, with a total scan time of 21.2 minutes per time point.

DTI images were transferred and processed offline using the diffusion tools available in the *fsl* suite (FMRIB, Oxford, UK). Volumes were manually cropped to exclude any non-brain voxels and decrease computation times. All the volumes over time were concatenated into a large four-dimensional file which was motion- and eddy-current corrected by linearly registering each frame to the first acquisition (t_0, b=0 s/mm^2; anesthetized animal) with 12 degrees of freedom. The corresponding diffusion gradient directions were rotated according to the obtained transformation matrices [5]. Next, the large 4-D volume was split into its components over time (7-8 time points) and DTI parameters (i.e., fractional anisotropy, FA and mean diffusivity, MD) were computed for each time point (Figure 1). Eight regions of interest (ROI) were manually drawn on the MD maps at t_0 and subsequently used to extract the DTI parameters of the voxels contained therein over the entire time series. The ROIs were placed on the corpus callosum and the sub-cortical gray matter, as well as on the left/right internal capsules, hippocampus dorsalis and hippocampus ventralis.

Changes in DTI paramers due to time after death were investigated. In order to remove the contribution that temperature plays on MD, we used the values reported by Holz et al. [4]. Within the temperature range of interest for our study (20-40 °C), such data can be adequately fit using a mono-exponential function. As MD measured in tissue is greatly different from that measured in free-diffusing water, analyses were performed based on the percent difference values between each time point and t_0; for the case of the free water model, the baseline diffusivity is considered to be at T=40 °C. Deviations from this model were attributed to tissue changes.

3 Results and Discussion

Diffusion anisotropy remained rather constant through time with only mild fluctuations from the baseline values for all three rabbits (Figure 2A). On the other hand, MD showed a progressive decrease that was very similar between all three rabbits (Figure 2B). The other white matter structures studied behaved in a very similar fashion. The hippocampi (in both their ventralis and dorsalis portions) showed a slight increase in FA over time (from 0.1±0.1 at baseline to 0.17±0.15 after 600 minutes), while MD decreased over time similarly to the white matter structures.

The changes in MD relative to t_0 were more pronounced than those expected based on the effects of temperature changes alone [4]. All three rabbits showed a progressive decline of MD values following death that continued until the end of the experiment (Figure 3). However, the deviation from the decay due to temperature alone seems to level-off after the first three hours. The same evolution was observed on the gray matter structures.
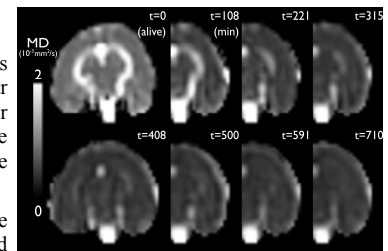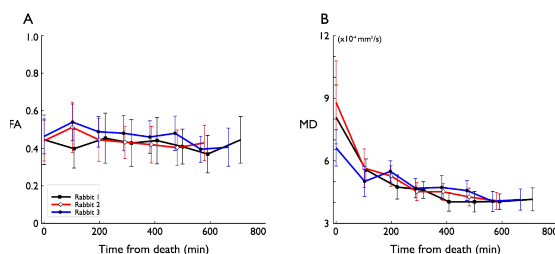
In a previous study, the diffusion characteristics of the mouse spinal cord were followed after death for ten hours with the cord in situ and at ambient temperatures [5]; diffusion anisotropy was found to be stable following death within the time frame studied, while water diffusivity decreased 50-60%. However, the time course of such decline of diffusivity was not clear, and whether or not it was related to tissue modifications, temperature, or both. Shepherd et al. [3] examined such a time course, by performing immersion fixation of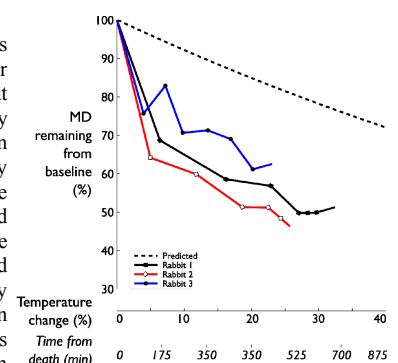 rat brain slices following different post-mortem intervals (0-24 h), and also found a progressive decline of water diffusivity as a function of post-mortem interval. Within the first few hours of degeneration, brain tissue showed increasing vacuolization, while the later stages (after 12 hours) there showed cell autolysis. Such methodology may underestimate the diffusion and tissue changes (compared to tissue degradation in situ). In a similar study, MD markedly decreased within the first few hours, although the mere fact of fixation seemed to decrease both FA and MD by 10-15% [6].

Figure 1

Figure 2

Figure 3

In conclusion, we found a considerable decrease of MD (with minimal changes of FA) of *in situ* brain tissue within the first three hours after death that cannot be attributed to temperature changes only and may be related to tissue degeneration, such as vacuolization and cellular swelling. In the later stages of degeneration, MD continued to decrease, although at this stage there seems to be an interplay between continuing temperature decrease (thus lowering MD) and gross cytoarchitectonic changes (such as autolysis) that can lead to increased diffusivity.

[1] D'Arceuil and de Crespigny, *NeuroImage* 36, 2007.
[2] Widjaja et al., *MRI* 27, 2009.
[3] Shepherd et al., *NeuroImage* 44, 2009.
[4] Holz et al., *Phys Chem Chem Phys* 2, 2000.
[5] Kim et al., *NMR Biomed* 20, 2007.
[6] Landman et al., *Proc. ISMRM-ESMRMB* p. 328, 2007.