Longitudinal changes in diffusivity after long-term storage of postmortem tissue

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Introduction

Diffusion weighted imaging (DWI) obtained postmortem is potentially superior to in vivo DWI. It resembles in vivo neuroanatomy and benefit from improved signal-to-noise ration (SNR) and spatial resolution through use of high-field MR scanners and longer scanning times. Moreover, degrading effects on DWI observed in vivo such as physiological noise are absent. The potential benefits of postmortem tissue for diffusion imaging include development, testing and validation of e.g. tractography algorithms [1], and the study of detailed anatomical micro structures not easily detected on clinical MR scanners. Furthermore, postmortem tissue allows long-term storage and hence repeated use in projects employing different imaging strategies. However, several issues need to be addressed to ensure that post-mortem DWI data are representative of in vivo DWI including I) tissue degeneration due to autolysis (cells that begin a self-degeneration process due to the action of their own autogenous enzymes) [2, 3], II) the consequences of low environmental temperature compared to in vivo, III) the influence of the fixative used [4], and finally IV) time-varying artefacts caused by e.g. physical handling of the tissue before scanning. We can overcome the above issues by using (transcardial) perfusion fixation to minimise tissue degeneration (issue I) and higher b-values [5] than typically used in vivo. The latter compensates for the slower molecular motion within postmortem tissue (issue II+III). Moreover, by introducing a resting period after positioning the brain in the scanner but before obtaining DWI datasets time-varying artefacts can be reduced (issue IV). Although solutions to the above exist, the stability of longitudinal diffusivity measures of postmortem tissue is still unknown. Therefore, the aim of the present study was to investigate the longitudinal stability of diffusivity measures during long-term storage of postmortem tissue. The porcine brain was used as an animal model for the human brain. The above four issues were addressed to ensure the quality of postmortem tissue. and repeated diffusivity measures were collected over a period of nearly four years.

Method

Three normal pig brains were used; two young Göttingen mini pigs (P1 and P2; 3 months of age, 5 - 6 kg in body weight) and one (P3) fully-grown landrace pig (>12 months, body weight 40 kg). The animals were sedated using a Zoletile® mixture and perfusion-fixated transcardially in 4% paraformaldehyde (PFA) [1]. This fixation procedure ensured a minimal introduction of autolysis in the tissue. Brains were removed, post-fixated for at least 12 hours in 1% PFA and then placed at 5°C for long-term storage. All procedures followed guidelines for the care and use of experimental animals approved by the Danish Animal Experiments Inspectorate.

An experimental 4.7T Varian Inova scanner was used to obtain diffusion weighted images. To reduce geometric image distortions and minimize susceptibility artefacts (e.g. due to air bubbles) a conventional diffusion weighted spin echo sequence was used. The b-values used were selected in the 4000 s/mm^2 range as described in [5]. Two (slightly different) acquisitions schemes were used: For P1 and P2: b-value = 4009 s/mm^2; voxel size: 0.51 x 0.51 x 0.5 mm^3. For P3: b-value = 4090 s/mm^2; voxel size: 0.63 x 0.63 x 0.6 mm^3. All DWI datasets consisted of 3 non-DW and 61 DW (non-collinear directions) image volumes. At least a slab of axial slices with the lowest slice starting from above the body of the corpus callosum was collected. Before acquiring DWI data a resting period of at least 15 hours after brain position ensured reduction of short-term instabilities from a time-varying artefact due to handling of the brain tissue and heat conduction. Two ROIs were semi-manually drawn on the non-DW image. A GM and a WM ROI included cerebral cortex and WM, respectively. Within each ROI mean diffusivity (MD) $(\lambda_1+\lambda_2+\lambda_3)/3$ and diffusivity along (λ_1) and perpendicular (λ_2 and λ_3) to the fibres was calculated using the tensor model. Since the WM ROI included a heterogeneous region of crossing fibres not correctly reconstructed by the single tensor model, only voxels with a FA value greater than 0.3 were included.

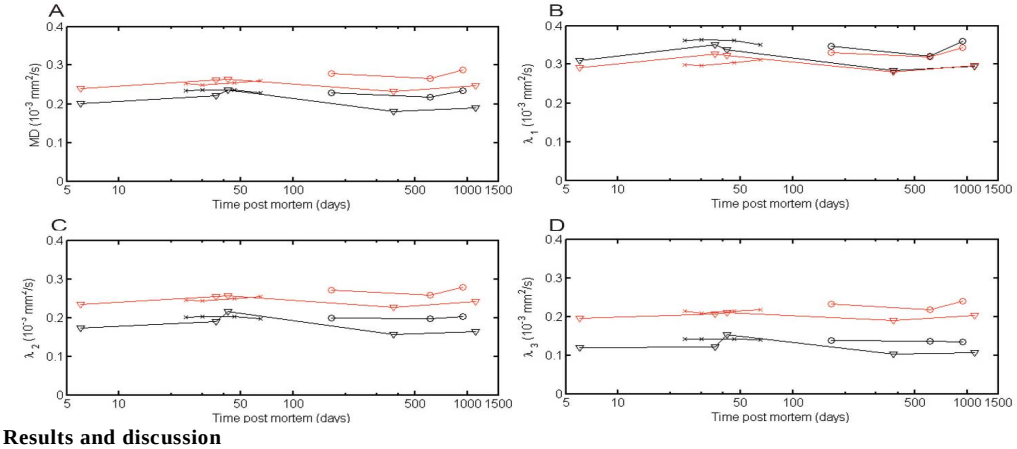

Fig. 1 Longitudinal changes in diffusivity of fixated postmortem brain tissue as a function of time post mortem (days) are shown: (A) MD and diffusivity along (B) and perpendicular (C, D) to the fibre bundles calculated within the GM ROI (red) and the WM ROI (black). Repeated measurements of P1(○), P2(x) and P3(▼).

Results and discussion

Throughout the investigation, no statistical differences in diffusivity were observed within or across brains in either the GM or WM ROI (Fig. 1). MD is comparable to those reported in other postmortem animal brain studies and is significantly decreased compared to in vivo (note that anisotropy in vivo and postmortem are comparable) [6,7,8]. However, minor variation in the longitudinal diffusivity is observed. Ongoing cross-binding of proteins due to the fixative used changes viscosity within the cellular spaces until final preservation of the tissue. The apparent plateau from days 25-80 post mortem might indicate attainment of this preservation stage, whereas later on (>100 days) diffusivity has stabilised at a lower level. The variation observed at these later time points (>100 days) might also be explained by minor chemical reactions ongoing within the tissue and other environmental factors. Our results (Fig. 1 B,C,D) suggest that the minor variations in diffusivity are orientation invariant because λ_1, λ_2 and λ_3 show a similar trend. This means that the variation is probably not related to changes in anatomical micro-structures, but more likely due to changes in the environment of the molecules In conclusion, long term-storage seems possible and, although minor variations in diffusivity might appear, not expected to significantly affect tissue structure. The unique possibility of long-term storage allows us to build up postmortem brain banks from which DWI datasets can be obtained with a standard specified by future imaging strategies.

References

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