Effects of formalin fixation on MR relaxation times in the human brain

Christoph Birkl[1], Christian Langkammer[2], Nicole Golob-Schwarzl[3], Marlene Leoni[3], Johannes Haybaeck[3], Walter Goessler[4], Franz Fazekas[1], and Stefan Ropele[1]
[1]Department of Neurology, Medical University of Graz, Graz, Austria, [2]MGH/HST Martinos Center for Biomedical Imaging, Harvard Medical School, Boston, MA, United States, [3]Department of Neuropathology, Institute of Pathology, Medical University of Graz, Austria, [4]Institute of Chemistry, Analytical Chemistry, University of Graz, Austria

Target audience

Researchers with interest in relaxometry and post mortem MRI.

Purpose

Post mortem MRI is an indispensable technique to validate quantitative MRI methods and to improve our understanding of relaxation mechanisms in tissue on a microscopic level. Among various fixation techniques, formalin fixation is the most frequently used technique for preserving the tissue samples from degradation.[1] However, formalin fixation was reported to significantly reduce T_1 and T_2 relaxation times which may hamper comparison with in vivo MRI.[2,3] Variations in temperature and fixation duration may additionally complicate the interpretation of post mortem MRI.[3,4] It has been speculated that the reduction of relaxation times originates from formalin induced cross-linking or dehydration,[5] but the actual cause and the magnitude of the concomitant T_1 and T_2 changes have not been investigated systematically so far. We here aimed at studying the origin and magnitude of T_1, T_2, and T_2* shortening in formalin fixed brain tissue by using water content measurements and electrophoresis.

Methods

Central 10mm thick brain slices from five deceased subjects were scanned at 3T, directly after autopsy (unfixed) and after 150 days of fixation with 4% formaldehyde (fixed). Relaxometry (T_1, T_2, T_2*) was performed at several temperatures ranging from 4°C to 37°C. From two additional brain slices, 2-3 samples were taken from white matter, cortex, and deep gray matter (= a total of 6-9 samples) and were split into two equally sized sub-samples for further analyses. The water content (WC) was calculated from the loss of mass after freeze drying and drying in an oven, according to WC = $(m_{wet} - m_{dry})/m_{wet}$ x 100. The molecular weight, as measure of the protein size and formalin induced cross-linking, was determined before and after formalin fixation using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis).

Results

After formalin fixation all relaxation times were significantly shortened (Table 1). The strongest effect was observed for T_1, which was reduced about 67% in white matter, 76% in the cortex and 67% in the basal ganglia compared to T_1 in the same unfixed sample. T_2 showed the strongest decrease in white matter and the lowest in the basal ganglia. The decrease of T_2* was approximately 28% in all regions. Overall, fixation caused only a small decrease of the WC. The highest decrease was observed in white matter with approximately 14% and the lowest in the cortex with approximately 0.5%. The decrease of WC in the basal ganglia was approximately 8%. All unfixed samples showed a typical distribution of the molecular weight over the entire range with a majority between 30 and 60 kDa (Figure 1). Cross-linked proteins of formalin fixed samples were greater than 260 kDa, visible at the loading slot only and therefore inseparable using SDS-PAGE.

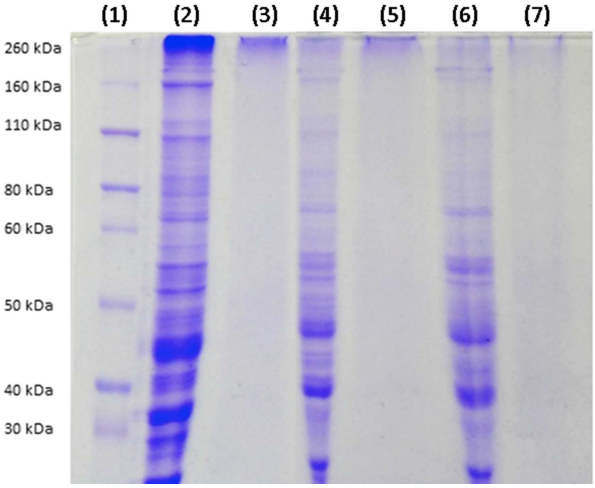

Figure 1: Representative SDS-PAGE gel of (1) standard, (2) cortex unfixed, (3) cortex fixed, (4) basal ganglia unfixed, (5) basal ganglia fixed, (6) white matter unfixed and (7) white matter fixed.

Tissue		Unfixed (ms)	Fixed (ms)	Relative decrease (%)
White Matter	T_1	822 ± 55	271 ± 109	67.0
	T_2	83.8 ± 24.8	62.1 ± 21	25.8
	T_2*	40.2 ± 10.3	28.6 ± 7.4	28.9
Cortex	T_1	1606 ± 264	384 ± 184	76.1
	T_2	140 ± 49	124 ± 40	11.1
	T_2*	46.7 ± 21.1	33.4 ± 16.3	28.5
Basal Ganglia	T_1	1223 ± 141	400 ± 153	67.3
	T_2	95.8 ± 35.1	90.2 ± 22.1	5.9
	T_2*	39.3 ± 15.6	29 ± 13	26.2

Table 1: Relaxation times (extrapolated to 37°C) of the unfixed tissue and after 190 days of formalin fixation.

Discussion and Conclusion

According to relaxation theory, T_1 and T_2 relaxation times are strongly coupled to WC. The substantial formalin induced T_1 and T_2 changes can therefore not be explained by the small change in WC solely. Formaldehyde reacts with water to methylene glycol, which interacts with protein side chains. This leads to crosslinking between the proteins through methylene bridges. The increased molecular size as a consequence of cross linking could be clearly demonstrated with gel electrophoresis. In particular T_1 is strongly related to the size of molecules and their resulting correlation times, i.e. larger molecules are less efficient in absorbing energy from relaxing water protons. Overall, our results clearly demonstrate that reductions in relaxation times are caused by formalin induced cross-linking while the WC is playing only a minor role. This has to be taken in to account when drawing conclusions from post mortem MRI.

Reference

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