The effects of tissue iron and temperature on R2* (=1/T2*) and ΔR2* contrasts

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Introduction

T_2^*-weighted images at ultra-high field (7T and above) have revealed large white matter (WM) contrast variation (1). The origins of this variation have been attributed to the differences in myelin content, microstructural shape and microstructural orientation (2-5). These findings have of great importance because they may open a new way of assessing WM integrity by measuring the changes in T_2^* or related contrasts. Recently, the relative orientation of WM fibers to B_0 has shown to change T_2^* measurement significantly (*e.g. in-vivo* human centrum semioval changed from 23.5 ms to 38 ms at 7 T) (6, 7). This B_0 orientation dependence has been exploited to generate a new fiber orientation map and a ΔT_2^* map, which is a measure of voxel-wise T_2^* change (T_2^* parallel to B_0 - T_2^* perpendicular to B_0) (5). The origin of this B_0 orientation dependency has been suggested as myelin in WM (5) and, if proven, the ΔT_2^* map may be used as a biomarker for myelin. However, it has not been shown whether tissue iron, which is another source of tissue susceptibility in brain (8, 9), has any contribution to this T_2^* orientation dependency. Distribution of iron (ferritin)-positive cells in WM is patchy but they are found to be aligned in rows (10). Therefore, it is plausible that iron may have certain influence on the B_0 orientation dependent T_2^* contrast. In this study, we investigated the contribution of tissue iron to WM R_2^* (= $1/T_2^*$) and ΔR_2^* (= R_2^* parallel to B_0 - R_2^* perpendicular to B_0). Instead of T_2^*, we used R_2^* because R_2^* has additive property for magnetic susceptibility sources (*i.e.* $R_2^* = R_2 + R_2'$). Additionally, the contribution of temperature to R_2^* and ΔR_2^* contrasts was explored since a few papers on magnetic susceptibility contrasts used fixed brain specimens to infer *in-vivo* results with no consideration of temperature difference.

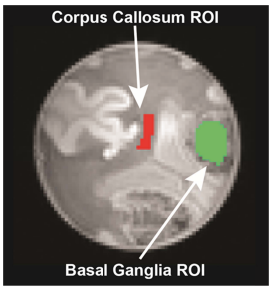

Fig.1: Specimen and ROIs

Methods

Temperature: To measure the contribution of temperature to R_2^* and ΔR_2^* contrasts, a specimen, a coronal slab of formalin-fixed human brain, was washed in PBS and scanned at a room temperature (21°C). Scanning was performed at two orientations: one with the corpus callosum fibers parallel to B_0 ($R_2^*(0°)$) and the other perpendicular to B_0 ($R_2^*(90°)$). A multi-echo 3D GRE sequence was used to measure R_2^*. The scan parameters were TR = 75 ms, TE = 4:4:36 ms, FA = 19°, resolution = 1 x 1 x 1 mm^3, matrix size = 128 x 128 x 16, and scan time = 155 s. After the scan, the container (with the specimen inside) was heated up to 38°C in a water bath. The temperature was monitored using a thermometer placed in contact with the specimen. Once the temperature reached 38°C, the container was removed from the water bath and stored in a custom-made heat shield and scanned immediately. The total scan time including localization and shimming was less than 4 min. The temperature was measured right after the scan and showed less than 2°C drop. This procedure was repeated to measure both $R_2^*(0°)$ and $R_2^*(90°)$ at 37°C. Two ROIs, corpus callosum and basal ganglia, were used for analysis (Fig. 1).

Tissue iron: To estimate the contribution of tissue iron on B_0 orientation dependent R_2^* contrast, we used iron chelating solution (1mM desferrioxamine and 2mM sodium dithionite) to remove iron from the specimen. This solution has been successfully used to remove iron from fixed tissues (9). The brain specimen was store in the solution on day 0 and the solution was replaced every 1 to 3 days. The R_2^* values were measured on day -4, 2, 4, 6, 8, 10, 12 and 14 to check the iron extraction induced R_2^* changes. ΔR_2^* (= $R_2^*(90°)$ - $R_2^*(0°)$) were measured on day -4 (pre-extraction) and day 14 (post-extraction). The same ROIs, shown in Fig. 1, were used to measure R_2^* and ΔR_2^* changes. The same MRI scan parameters as above were used for data acquisitions.

Temperature		21°C	37°C
Corpus Callosum	$R_2^*(0°)$	51.4 ± 3.1 Hz	47.4 ± 3.0 Hz
	$R_2^*(90°)$	47.5 ± 2.7 Hz	41.5 ± 2.1 Hz
	ΔR_2^*	3.9 ± 2.9 Hz	5.9 ± 2.6 Hz
Basal Ganglia	$R_2^*(0°)$	67.1 ± 8.9 Hz	58.3 ± 8.9 Hz
	$R_2^*(90°)$	66.6 ± 8.0 Hz	58.7 ± 7.5 Hz
	ΔR_2^*	0.6 ± 8.4 Hz	-0.2 ± 8.1 Hz

Table 1: Effects of temperature

Results

Temperature effects (see Table 1): Temperature increase from 21°C to 37°C showed decrease in R_2^* values (increase in T_2^*) in all four measurements (two ROIs x two orientations, $p < 0.01$). This decrease is consistent with lower R_2^* values observed *in vivo* (corpus callosum: 35.7 to 32.3 Hz (1) and basal ganglia: 49.3 to 50.7 Hz (11)) compared to fixed brains suggesting that a certain portion of decrease in R_2^* values originates from the temperature difference. ΔR_2^* change was statistically significant only in corpus callosum ($p < 0.01$) which agrees with the previous observations that only WM shows B_0 orientation dependence. The ΔR_2^* values in corpus callosum was increased from 3.9 Hz at room temperature to 5.9 Hz at body temperature ($p < 0.01$) (see Discussion).

Tissue iron effects (see Table 2): Figure 2 shows R_2^* change over the two weeks of the iron extraction period. The R_2^* values were significantly reduced over time indicating that the contribution of iron on R_2^* in both ROIs was significant. Basal ganglia, which are known to have large iron concentration, show much larger reduction in R_2^*. When B_0 orientation dependent R_2^* contrast was compared, the values in corpus callosum show little change from 3.9 Hz to 4.4 Hz (non-significant for $p < 0.01$). This finding suggests that iron may not contribute significantly to the B_0 orientation dependent R_2^* contrast.

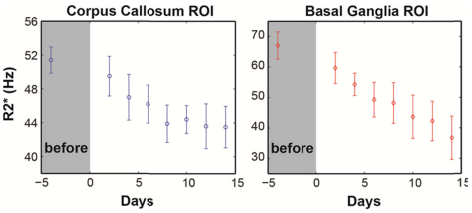

Figure 2: R_2^* change in iron extracting solution

Iron		Before	After (day 14)
Corpus Callosum	$R_2^*(0°)$	51.4 ± 3.1 Hz	43.5 ± 4.9 Hz
	$R_2^*(90°)$	47.5 ± 2.7 Hz	39.1 ± 3.5 Hz
	ΔR_2^*	3.9 ± 2.9 Hz	4.4 ± 4.3 Hz
Basal Ganglia	$R_2^*(0°)$	67.1 ± 8.9 Hz	36.8 ± 14.2 Hz
	$R_2^*(90°)$	66.6 ± 8.0 Hz	36.4 ± 14.2 Hz
	ΔR_2^*	0.6 ± 8.4 Hz	0.5 ± 14.2 Hz

Table 2: Effects of iron extraction

Discussion and Conclusion

In this study, we investigated the effects of tissue iron and temperature on R_2^* and B_0 orientation dependent R_2^* (ΔR_2^*) contrasts. The results show that iron has large effects on R_2^* but has limited effects on ΔR_2^* suggesting other susceptibility source(s) is responsibility for the ΔR_2^* contrast. As suggested before, we suspect myelin as a primary source for this contrast because of its highly oriented structure and large susceptibility value (12, 13). Temperature is an important compounding factor in translating in-vitro findings to in-vivo. Our study suggests that temperature has contribution on both R_2^* and ΔR_2^* contrasts. This temperature effect may originate from temperature-dependent diamagnetism. It is known that the susceptibility of diamagnetic material is temperature dependent. For example, water susceptibility is linearly dependent on temperature (χ = -9.032 ppm at 20°C and -9.054 ppm at 37°C) (14) and different materials have different temperature dependency. Further investigation is necessary to measure temperature dependent myelin susceptibility to confirm the origin of temperature dependent R_2^* and ΔR_2^* change in WM.

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