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Blood Oxygen Level Dependent Magnetization Transfer (BOLDMT) Effect

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Abstract

A few studies have reported that magnetization transfer (MT) preparation interacts with blood oxygen level dependent (BOLD) contrast used for functional magnetic resonance imaging (MRI). However, the mechanism is still not well established. This study shows that blood oxygenation level itself affects MT contrast. MT ratio (MTR) decreases with increased blood oxygenation, which is demonstrated by ex vivo and in vivo experiments. Oxygenated blood shows less MTR contrast compared to deoxygenated blood sample; and higher levels of oxygen inhalation decrease tissue MTR in vivo especially in brain tumor region. The percentage reduction of MTR due to hyperoxia inhalation, referred to as the blood oxygen dependent magnetization transfer (BOLDMT) effect, correlates well with tissue oxygen extraction, which is highest in well-vascularized tumor rim, followed by inner tumor, gray matter (GM), and white matter (WM) normal tissue. Simulations and experiments demonstrate that BOLDMT effect induced with hyperoxia inhalation may be generated by decreased tissue T_1 due to increased O_2 dissolution and increased tissue T_2 due to reduced deoxyhemoglobin (dHb) concentration. Compared to regular T_2^* weighted BOLD contrast, BOLDMT has higher insensitivity to B_0 inhomogeneities. BOLDMT may potentially serve as a reliable and novel biomarker for tumor oxygen extraction.

Keywords

Blood oxygen level dependent; Magnetization transfer; Tumor oxygen extraction; Hyperoxia inhalation

1 Introduction

Conventional magnetization transfer (MT) in magnetic resonance imaging (MRI) provides a unique contrast associated to bound protons [1, 2]. Saturating macromolecular bound protons with off-resonance radio frequency (RF) pulses creates MRI detectable signal change from the bulk water protons due to magnetization transfer between the macromolecular protons and bulk water. Blood oxygenation level dependent (BOLD) imaging [3], typically based on T_2^* weighted gradient echo signals, provides contrast from

paramagnetic deoxyhemoglobin (dHb) in the blood. Increases in oxygenated hemoglobin concentration leads to increased T_2^* or decreased R_2^* ($1/T_2^*$), higher BOLD-MRI signals. This concept has been widely used in MRI of oxygenation in different tissues including brain [3], kidney [4], and tumors [5]. However, regular T_2^* weighted BOLD MRI is prone to artifacts associated with B_0 field inhomogeneity and fluctuations [6, 7].

MT and BOLD contrasts could interact with each other. Zhou et al. investigated the MT effect in rat brain at a range of arterial pCO₂ levels [8] and showed an altered MT response in the brain due to varied arterial pCO₂. In another study, functional MRI with MT preparation has been proposed for quantification of arterial cerebral blood volume (CBVa) alternation [9]. However, the fundamental mechanism on how MT contrast is affected by functional activation and blood oxygenation is still not clear.

Our hypothesis is that blood oxygenation level itself could directly affect MT contrast. Blood oxygen level dependent magnetization transfer (BOLDMT) effect is demonstrated in this study with stationary ex vivo blood experiments and tumor rats subjected to different percentages of oxygen inhalation. The advantage of BOLDMT over regular BOLD MRI is demonstrated.

2 Methods

Fresh rat blood samples with heparin are bubbled with 100% N₂ or 100% O₂ for 10 min at a rate of 2 cc/min and imaged on a 7 T Siemens whole-body MRI scanner with a 35 mm-diameter custom-built solenoid coil. A custom pulse sequence was written to provide a frequency selective saturation pulse followed by a segmented RF spoiled gradient echo (GRE) readout (128 segments per shot for every 10 s). Sequence parameters used were: slice thickness = 5 mm, flip angle = 10°, readout TR = 12.2 ms, TE = 6 ms, in-plane resolution = $270 \times 270 \mu\text{m}^2$. Z spectra between -100 p.p.m. and +100 p.p.m. were collected using ten Hanning-windowed rectangular pulses (100 ms each with 200 μs inter-delay) at the average B_1 of 250 Hz.

To develop intracranial tumors, rat gliosarcoma cells (9 L) were injected into Syngeneic female Fisher rats (F344/NCR, 4–6 weeks old) weighing 130–150 g, as reported previously [10, 11]. At 4–6 weeks after tumor implantation, brain tumor-bearing rats ($n = 5$) were scanned at a Varian 9.4 T horizontal bore small animal scanner and placed in a 35 mm diameter commercial quadrature proton coil. Z spectra of an axial slice of rat brain were acquired from rats subjected to different levels of inhalation oxygen (8, 21, 40, 60, 80, 100% diluted with N₂), at a fixed flow rate of 35 cc/min. After any specific gas had been given for 3 min, to allow blood oxygenation to reach a steady state, Z spectra (100, -/+20, -/+10, -/+5, -/+4, -/+3.5, -/+3, -/+2.5 ... 0 p.p.m.) were collected using a similar customized sequence as mentioned except using 1 s continuous rectangle saturation pulse with 250 Hz B_1 slice thickness = 2 mm, flip angle = 15°, readout TR = 6.2 ms, TE = 2.9 ms, and 64 segment acquisition per shot for every 4 s (saturation pre-delay time = 3 s). Following Z spectra acquisition, T_2^* -weighted images were acquired using a gradient echo sequence at various TE (3, 5, 7.5, 10, 15, and 20 ms), from which R_2^* maps were generated. B_0 field map of the same slice was also generated with short TEs (2.5, 3.0, and 3.5 ms) [12]. All

animal experiments were performed according to an approved institutional animal care and use committee protocol.

Magnetization transfer ratio (MTR) contrast depends on various physiological parameters including macromolecular concentration, bound proton exchange rate, tissue T_1 and T_2 . MT effect as a function of bulk water T_1 (1–2.5 s) and T_2 (20–80 ms) was simulated using the Bloch equations modified for two-pool chemical exchange model (Bloch-McConnell equations) [13]. Other parameters are exchange rate = 10 Hz, free and bound proton concentration = 110 M and 4 M, respectively, bound proton offset = –2.4 p.p.m., bound proton $T_1/T_2 = 1$ s/10 ms. Saturation pulse parameters are the same as those in the blood experiments at 7 T. MTR as a function of field fluctuation was simulated by shifting B_0 static field between ± 1 p.p.m.

Image processing was performed using MATLAB. MTR spectrum is the plot of $1 - \text{MT}_{\text{on}}/\text{MT}_{\text{off}}$ as a function of the saturation offset, where MT_{on} and MT_{off} are the signals with and without saturation, respectively. Specifically for generating MTR maps, +20 p.p.m. signal was used for MT_{on} and 100 p.p.m. for MT_{off} , since it preserves over 99% of signal. BOLDMT contrast represented the percentage MTR change induced by hyperoxia inhalation compared to the baseline under normoxia. Regular BOLD contrast is generally calculated as the relative changes of T_2^* weighted signal or R_2^* ($1/T_2^*$).

3 Results

Blood oxygen saturation reaches 100 and ~80% after being bubbled with 100% O_2 and 100% N_2 respectively, under these experimental conditions. Signal at a given saturation offset from fully oxygenated blood is generally higher than deoxygenated blood, presumably due to regular BOLD effect (Fig. 5.1a). MTR spectrum (Fig. 5.1b) is broader in deoxygenated blood. As a result, MTR at +20 p.p.m. of deoxygenized blood is (20.1 $\pm$ 2.6%) higher (Fig. 5.1c).

Z spectra of a brain tumor-bearing rat subject to different oxygen inhalations are shown in Fig. 5.2a. MTR spectral line-width of tumor increases as the inhalation oxygen decreases, while the line-widths of gray matter (GM) and white matter (WM) are much less sensitive to oxygen inhalation (Fig. 5.2b). Relative MTR contrast at +20 p.p.m. under different oxygen inhalations are plotted in Fig. 5.2d, showing tumor rim and tumor has >4 times MTR change induced by hyperoxia inhalation comparing to the normal brain tissues. In the +20 p.p.m. MTR maps (Fig. 5.2c, top), lowest MTR contrast was seen from regions with high vessel density (mostly tumor rim), followed by tumor, GM, and WM. Tumor MTR decreases slightly under hyperoxia inhalation. BOLDMT map (Fig. 5.2c, bottom) shows highest contrast from tumor rim followed by tumor, GM, and WM. However, T_2^* -weighted and R_2^* contrast maps (Fig. 5.2f, g) are highly modulated by B_0 field (Fig. 5.2) with over 0.5 p.p.m. variation.

4 Discussions

Our experimental data in phantom and in vivo have demonstrated the changes in MTR due to changes in blood oxygenation. Increased supply of oxygen reduces blood dHb concentration, which primarily raises T_2 and T_2^* [14–16]. Additionally, excess O_2 dissolves into tissue, primarily reducing the T_1 of tissues [15, 17]. Theoretical simulation demonstrated that both decreasing bulk water T_1 and increasing T_2 reduce MTR contrast (Fig. 5.3a, b).

MTR contrast is insensitive to field inhomogeneity with <5% of MTR variation under 1 p.p.m. field fluctuation (Fig. 5.3c), since MT preparation pulse has a large frequency offset away from the center water resonance. In comparison, regular BOLD contrast is highly sensitive to B_0 homogeneity [7,18] as demonstrated in vivo (Fig. 5.2f, g).

In general, tumors have higher metabolism compared to normal tissue. Increased oxygen inhalation may provide more oxygen to tumor tissue and increase the tissue oxygen extraction. Tumor BOLDMT contrast map due to hyperoxia inhalation matches the expected oxygen extraction ratio of brain tumor by showing the highest magnitude in tumor rim and then inner tumor tissue, followed by GM and WM, suggesting that BOLDMT could be used as an indicator of tumor oxygen extraction with hyperoxia inhalation. Oxygen-sensitive electrodes [19–21] and histological staining of hypoxia biomarker, such as EF5 [22], may help us to establish the correlation between BOLDMT map and tumor oxygen extraction ratio in the future work. Future research on the contributions of blood flow and saturation pre-delay time on the in vivo BOLDMT effect would help us to fully understand and optimize BOLDMT contrast.

Imaging techniques for tumor oxygenation, characterized by hypoxia, have been reviewed [23]. So far, there is no reliable method for MRI of tumor oxygenation. BOLDMT effect based on hyperoxia inhalation might be used in clinical studies for imaging pathologies with altered oxygenation, such as tumor, Alzheimer's disease, and stroke.

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Fig. 5.1.

Ex vivo experiments on stationary blood samples. Z spectra (**a**) and MTR spectra (**b**) of blood samples bubbled with 100 % O_2 (red) or 100 % N_2 (blue). Corresponding MTR maps at +20 p.p.m. and percentage MTR change are shown in (**c**)

Fig. 5.2.

Representative Z spectra (a) and MTR spectra (b) from tumor and normal brain tissues under different levels of oxygen inhalations. (c) +20 p.p.m. MTR (top) and BOLDMT (bottom) maps of the same slice. Tumor BOLDMT contrast (bottom right) was color-coded over the anatomical image in gray scale. (d) Relative MTR contrast (mean $\pm$ standard deviation, $n = 5$) at different oxygen inhalations. (e) B_0 field map. (f and g) Relative T_2^* -weighted (TE= 10 ms) and R_2^* changes due to hyperoxia inhalation

Fig. 5.3.

Relative MTR at +20 p.p.m. as a function of bulk water T_1 (a) and T_2 (b) and static B_0 field fluctuation (c)