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Amide proton transfer imaging with improved robustness to magnetic field inhomogeneity and magnetization transfer asymmetry using Saturation with Frequency Alternating RF Irradiation (SAFARI)

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Abstract

Amide proton transfer (APT) imaging has shown promise as an indicator of tissue pH and as a marker for brain tumors. Sources of error in APT measurements include direct water saturation, and magnetization transfer (MT) from membranes and macromolecules. These are typically suppressed by post-processing asymmetry analysis. However, this approach is strongly dependent on B_0 homogeneity and can introduce additional errors due to intrinsic MT asymmetry, aliphatic proton features opposite the amide peak, and radiation damping-induced asymmetry. Although several methods exist to correct for B_0 inhomogeneity, they tremendously increase scan times and do not address errors induced by asymmetry of the z-spectrum. In this paper, a novel saturation scheme - saturation with frequency alternating RF irradiation (SAFARI) - is proposed in combination with a new magnetization transfer ratio (MTR) parameter designed to generate APT images insensitive to direct water saturation and MT, even in the presence of B_0 inhomogeneity. The feasibility of the SAFARI technique is demonstrated in phantoms and in the human brain. Experimental results show that SAFARI successfully removes direct water saturation and MT contamination from APT images. It is insensitive to B_0 offsets up to 180Hz without using additional B_0 correction, thereby dramatically reducing scanning time.

Keywords

APT – Amide proton transfer imaging; CEST – chemical exchange saturation transfer; SAFARI – saturation with alternating frequency RF irradiation; MT – magnetization transfer

Introduction

Chemical Exchange Saturation Transfer (CEST) imaging (1–3) is a method to detect molecules with exchangeable protons by saturating the exchangeable resonance and then observing the effects of the saturation transferred to water by exchange. This technique is of interest both for detecting particular molecules or types of molecules and also as a measure of pH, since the exchange rate of protons is often pH sensitive (4,5). Among the stronger *in-vivo* contributions to CEST contrast are hydroxyl protons at approximately 1ppm above water, that have been used to assess glycogen (6) and glycosaminoglycans (7) in tissues

where these molecules dominate, rapidly exchanging amine protons of amino acids and other molecules between 2–3 ppm above water, and the more slowly exchanging amides on the backbone of proteins, at approximately 3.5 ppm. Because amide protons have a larger chemical shift and their slow exchange permits saturation at power levels more compatible with human applications, amide proton transfer (APT) imaging (8,9) is particularly attractive for pH measurement with endogenous CEST contrast.

At pH values occurring *in-vivo*, the chemical exchange of amide protons is base-catalyzed (10), therefore APT contrast decreases with decreasing intracellular pH. This has been exploited for the study of acute cerebral ischemia (8,11–16). It has been shown that the APT contrast correlates with pH (8,13) and may help distinguish benign oligemia from the ischemic penumbra (14). As a result, APT imaging could become an important marker to predict progression to infarction in stroke.

In addition to being sensitive to pH, APT contrast is also related to the protein content inside cells, although it is not yet clear which specific proteins in which compartments contribute to the signal. Nevertheless, this property of APT imaging has been exploited for the study of tumors (17–22), multiple sclerosis (23,24) and to design a CEST reporter gene to map a specific gene expression pattern (25). In cancer imaging, APT imaging appears to correlate with tumor grade (21) and may distinguish active tumor regions from edema and normal-appearing white matter (18), as well as from necrotic tumor cores (20,26). APT imaging has also shown potential to differentiate between tumor recurrence and radiation necrosis (22), which otherwise appear identical on radiological images.

Although, APT imaging has emerged as a new source of MRI contrast with several promising clinical applications, including stroke and cancer imaging, it has so far been limited to research use due to the difficulty of measuring the APT effect *in-vivo* without errors. The RF irradiation needed to generate APT contrast also induces direct water saturation and magnetization transfer (MT) from semisolid protons in membranes and macromolecules. These effects both decrease the signal of the APT-weighted image, confounding the APT measurement. The standard method to correct for direct water saturation and MT is MT ratio asymmetry (MTR_{asym}) analysis, where the APT image is subtracted from a control image acquired with RF irradiation at the frequency opposite the amide protons with respect to the water line. This method, however, is dependent on the water frequency being centered and leads to severe artifacts when B_0 is not homogeneous, as occurs *in-vivo*.

Errors induced by B_0 inhomogeneity can be corrected to generate APT maps free of B_0 artifacts. This is typically accomplished by acquiring a z-spectrum and recentering the water line before performing the asymmetry subtraction. Because the z-spectrum involves acquiring many images with a range of RF irradiation frequencies (20–40 offsets), this method increases scan time beyond what is suitable for clinical application. Several alternative B_0 correction methods have been developed to reduce scan time. These include variations on the z-spectrum method (21,26,27), multiple offset acquisitions with additional B_0 mapping (28,29), and fitting of an analytical model (30). Yet, even with B_0 correction asymmetry analysis introduces additional errors in the APT measurement due to asymmetries in the z-spectrum.

Several sources of asymmetry of the z-spectrum – other than amide protons – contribute to the MTR_{asym} measurement, introducing errors in the quantification of the amide proton signal. First, aliphatic protons that have resonances in the range from –1.3ppm to –4.8ppm (8,9) can decrease the signal of the control image by saturation transfer. At low RF power, this tends to cancel out the amide proton signal in the MTR_{asym} calculation (31). Second,

probe detuning can cause radiation damping-induced asymmetry of the z-spectrum (32) introducing additional errors in MTR_{asym} . Third, the center of symmetry of the MT line is shifted with respect to the water line (33–35) and, therefore, does not completely cancel in the asymmetry subtraction. Again, this will cancel out the amide proton signal leading to MTR_{asym} values near zero or negative. Recently, an approximate analytical model (36), and sophisticated alternative acquisition methods (37,38) have been proposed to separate the amide proton signal from MT.

In this paper, we propose a novel APT acquisition method using saturation with frequency alternating RF irradiation (SAFARI) along with a new magnetization transfer ratio= MTR_{SAFARI} to remove direct water saturation and MT from APT images without additional B_0 correction. This technique was first tested in phantoms including a control relaxation phantom without chemical exchange in order to establish that SAFARI does not introduce contrast based on T_2 rather than CEST, and a bovine serum albumin protein phantom to test the sensitivity of SAFARI to amide proton exchange and aliphatic proton contamination. The phantoms were also imaged with increasing RF frequency offsets to test the range at which MTR_{SAFARI} remains insensitive to B_0 shifts while still saturating amide protons. The performance of APT-SAFARI in the presence of CEST and MT was then evaluated in a set of experiments performed in humans. High Signal-to-Noise Ratio (SNR) APT images of the brain were acquired to compare MTR_{asym} and MTR_{SAFARI} maps without B_0 correction. Lower SNR APT images were also acquired at 51 frequency offsets, akin to a z-spectrum, in order to compare both the B_0 and MT sensitivity of MTR_{asym} with B_0 correction and MTR_{SAFARI} without B_0 correction. Finally, the sensitivity of MTR_{SAFARI} to chemical exchange was studied by varying the average saturation power, similar to the “quantification of exchange as a function of saturation power” (QUESP) technique (39).

Theory

The key to optimizing APT contrast is achieving full amide proton saturation while minimizing the effects of direct water saturation and MT. We propose a novel scheme to produce amide proton transfer contrast based on saturation with frequency alternating RF irradiation (SAFARI). The APT-SAFARI technique relies on the non-linearity of the saturation process: once the amide proton line has been fully saturated, increasing the RF irradiation power should not affect the saturation significantly. Therefore, if the applied RF power is sufficient to produce complete amide proton saturation, the APT effect becomes independent of power. The broad macromolecular line and the water line, however, are not fully saturated. As a result, the effect of direct water saturation and MT vary mostly linearly with power. The APT-SAFARI acquisition and post-processing method exploits this different power dependence of the amide proton line compared to the water line to cancel out the effects of MT and direct water saturation, even in the presence of B_0 inhomogeneity and MT asymmetry, as described below.

The SAFARI image is acquired with RF irradiation applied with equal power at both the control (−3.5ppm) and label (+3.5ppm) frequencies. This is achieved within a pulsed-RF irradiation module by alternating every other saturation pulse between the two frequencies. If the total RF power applied at 3.5ppm is sufficient, the amide proton line will be fully saturated. Therefore, the signal in the SAFARI image can be modeled as a combination of the chemical exchange saturation transfer (CEST), as well as signal loss due to direct water saturation (W) and magnetization transfer (MT) from both off-resonance frequencies:

$$S_{\text{sat}}(\text{SAFARI}) = S_0 - \text{CEST} - P * W(|\omega_s + \delta_{B_0}|) - P * W(|-\omega_s + \delta_{B_0}|) - P * MT(|\omega_s + \delta_{MT} + \delta_{B_0}|) - P * MT(|-\omega_s + \delta_{MT} + \delta_{B_0}|) \quad [1]$$

where S_0 is the unsaturated reference signal intensity, δ_{B_0} is the B_0 inhomogeneity, δ_{MT} is the center of MT asymmetry, $\omega_s=3.5\text{ppm}$ is the amide proton frequency and P represents the RF irradiation power. In the presence of B_0 inhomogeneity, such as susceptibility effects *in-vivo*, the water line is shifted and direct water saturation is no longer symmetric around 0ppm, taken into account by the δ_{B_0} variable. The CEST effect, however, is independent of δ_{B_0} as long as the RF saturation bandwidth is broad enough to maintain full amide proton saturation despite the shift of the line. The center of MT symmetry is known to be intrinsically shifted with respect to the water line, and is represented here by δ_{MT} . The SAFARI image, therefore, contains the individual asymmetric effects of direct water saturation and MT at both the positive and negative saturation frequencies.

In addition, we acquire the standard CEST image with RF irradiation of the amide proton line only. This is achieved by applying all of the RF pulses of the saturation module at the label frequency only. As a result, the total RF power deposited on the amide proton line is doubled compared to the SAFARI image. Consequently, the MT and direct water saturation effects double, while the APT effect does not change:

$$S_{\text{sat}}(+\omega_s) = S_0 - \text{CEST} - 2P * W(|\omega_s + \delta_{B_0}|) - 2P * \text{MT}(|\omega_s + \delta_{MT} + \delta_{B_0}|) \quad [2]$$

Similarly, the signal in the control image with all RF pulses applied at the control frequency only can be modeled as:

$$S_{\text{sat}}(-\omega_s) = S_0 - 2P * W(|-\omega_s + \delta_{B_0}|) - 2P * \text{MT}(|-\omega_s + \delta_{MT} + \delta_{B_0}|) \quad [3]$$

The signal amplitudes resulting from the three different saturation preparations of the APT-SAFARI scan are illustrated in Figure 1.

The common approach for separating CEST contrast from other effects is asymmetry analysis, where the reference image is subtracted from the CEST image. The contrast generated by standard asymmetry analysis is given by ([3]–[2]):

$$\begin{aligned} MTR_{\text{asym}} &= MTR(+\omega_s) - MTR(-\omega_s) \\ &= [1 - S_{\text{sat}}(+\omega_s)/S_0] - [1 - S_{\text{sat}}(-\omega_s)/S_0] \propto S_{\text{sat}}(-\omega_s) - S_{\text{sat}}(+\omega_s) \\ &= \text{CEST} + 2P * \Delta\text{MT} + 2P * \Delta W \end{aligned} \quad [4]$$

MTR_{asym} fails to isolate the CEST contrast because direct water saturation and MT are not symmetric around 0ppm. Instead of the standard asymmetry analysis, we employ a three-way subtraction where the SAFARI image is doubled and subtracted from the sum of the positive and negative frequency images ([2]+[3]–2[1]):

$$\begin{aligned} MTR_{\text{SAFARI}} &= 2 * MTR(\text{SAFARI}) - MTR(+\omega_s) - MTR(-\omega_s) \\ &= 2 * [1 - S_{\text{sat}}(\text{SAFARI})/S_0] - [1 - S_{\text{sat}}(+\omega_s)/S_0] - [1 - S_{\text{sat}}(-\omega_s)/S_0] \propto S_{\text{sat}}(+\omega_s) + S_{\text{sat}}(-\omega_s) - 2S_{\text{sat}}(\text{SAFARI}) \\ &= \text{CEST} \end{aligned} \quad [5]$$

As a result, both MT and direct water saturation cancel out exactly, even in the presence of B_0 inhomogeneity and MT asymmetry.

Methods

APT Pulse sequence design

The APT-SAFARI imaging pulse sequence is shown in Figure 2. Four images are acquired for each APT-SAFARI scan: one label image $S_{\text{sat}}(\omega_+)$ with all saturation pulses applied at a single frequency ω_+ (Fig. 2a), one SAFARI image $S_{\text{sat}}(\text{SAFARI})$ with saturation pulses alternating between ω_+ and ω_- (Fig. 2b), one control image $S_{\text{sat}}(\omega_-)$ with all saturation pulses applied at a single frequency ω_- (Fig. 2c), and another SAFARI image $S_{\text{sat}}(\text{SAFARI}')$ with saturation pulses alternating between ω_- and ω_+ (Fig. 2d). Note that the first and third images constitute a standard CEST experiment. It was necessary to acquire two SAFARI images with reversed ordering of the pulses to ensure that identical RF powers were deposited on the positive and negative frequencies.

For all four images, the off-resonance pulsed-RF irradiation consisted of a 3 second train of Blackman shaped inversion pulses with pulse width (pw) = 9 ms repeated every $\text{TR}_{\text{RF}} = 15$ ms while incrementing the change in phase shift linearly by 117° for spoiling of transverse magnetization (40). The corresponding continuous wave (CW) RF power is given by

$B_{\text{CW}} = \frac{1}{\gamma \text{TR}_{\text{RF}}} \int_0^{\text{pw}} \gamma B_1(t) dt = 0.78 \mu T$ (41), where $B_1(t)$ is the Blackman waveform given by the MATLAB (Mathworks, Natick MA) “blackman” function. These pulse parameters were optimized to saturate the amide protons at 3.5ppm even in the presence of B_0 shifts, while within the constraint of not producing strong direct water saturation (42). Crusher gradients were turned on between every RF pulse to destroy remaining transverse magnetization. The saturation module was followed by a single-shot echo-planar acquisition described below.

Phantom preparation

Control phantoms were prepared with eight different concentrations of MnCl_2 in water. Each solution was divided amongst four 15mm-diameter centrifuge tubes. The tubes were arrayed in a single 4 by 8 Styrofoam holder for imaging. T_1 and T_2 relaxation times have been measured previously (43). T_1 values range from 2000ms to 400ms and T_2 values range from 280 ms to 40ms. A protein phantom was prepared by dissolving 5g of bovine serum albumin (BSA, Sigma-Aldrich) in 50ml phosphate saline buffer at pH=5.5. The BSA solution was transferred to a 29.1mm-diameter tube for imaging.

Imaging

All studies were performed on a 3T GE SIGNA EXCITE MR system using the body coil for transmit. A standard eight-channel receive-only head array was used for signal reception for both phantom and human imaging. A single shot spin-echo echo-planar imaging (EPI) readout was used for all control phantom and human studies with $\text{TR} = 4\text{s}$, TE between 63 and 64 ms, $\text{FOV} = 24\text{cm}$, matrix size = 96×96 , and slice thickness = 12mm. The BSA phantom was imaged with the same parameters, except for a $\text{FOV} = 15\text{cm}$ and $\text{TE} = 17.8$ ms.

The control phantoms were imaged with the long axis of the tubes along the direction of the magnetic field. A modified z-spectrum was acquired to test the robustness of $\text{MTR}_{\text{SAFARI}}$ against B_0 inhomogeneity. Shifts in the water line due to B_0 offsets were mimicked by introducing an offset f to the off-resonance irradiation frequency. Instead of acquiring APT scans with off-resonance irradiation at the amide proton frequency $\omega_s/2\pi = 450\text{Hz}$, the applied frequencies were $\omega_+ = 2\pi f + \omega_s$ and $\omega_- = 2\pi f - \omega_s$, where the frequency offset f was swept from -900Hz to $+900\text{Hz}$ in steps of 30 Hz. Therefore, the frequency shift f simulates an offset of the water line due to a B_0 shift of $-f$. The modified z-spectrum acquisition resulted in a total of 61 APT scans (244 images). Note that for the single frequency images

the data set constitutes a standard z-spectrum acquired out of order. For the SAFARI images, however, it differs from a z-spectrum because the offset f leads to RF irradiation at frequencies with increasing asymmetry around the water line, rather than symmetric frequencies with increasing offsets. Total scan time was 15 minutes for each phantom.

The BSA phantom was imaged with the long axis of the tube along the direction of the magnetic field. The amide proton resonance of BSA is located at 350Hz (see Figure 4b), therefore, the modified z-spectrum was acquired with applied frequencies $\omega_+ = 2\pi(f + 350\text{Hz})$ and $\omega_- = 2\pi(f - 350\text{Hz})$, where the frequency offset f was swept from -350 Hz to $+350\text{Hz}$ in steps of 14 Hz . The modified z-spectrum acquisition resulted in a total of 51 APT scans (204 images) in just under 14 minutes. Because the BSA amide resonance is 100Hz closer to the water resonance than the amide proton resonance in the brain, the experiment was also repeated with a modified RF irradiation module with $\text{pw} = 12\text{ ms}$ repeated every $\text{TR}_{\text{RF}} = 20\text{ ms}$ to provide a more selective saturation bandwidth and reduce direct water saturation..

Human imaging was performed following a protocol approved by the institutional review board. Four healthy volunteers (2 men, 2 women; age range 23–40 years, mean age 30 years) were scanned after giving written informed consent. For each volunteer, a single slice was located at the level of the ventricles. One unsaturated S_0 image was acquired for reference with 12 averages. One high SNR APT-SAFARI scan with 12 time-interleaved repetitions of the sequence (48 images total) was acquired with off-resonance irradiation at the amide proton frequency. Scan time was 3 minutes. In order to evaluate the robustness of $\text{MTR}_{\text{SAFARI}}$ against B_0 inhomogeneity, 3 high SNR APT-SAFARI scans were acquired with frequency offsets $f = 100, 150, \text{ and } 200\text{Hz}$, corresponding to RF irradiation at the frequency pairs $+550\text{Hz}/-350\text{Hz}$, $+600\text{Hz}/-300\text{Hz}$, and $+650\text{Hz}/-250\text{Hz}$, respectively. Scan time for the high SNR scans with frequency offsets was 3 minutes each for a total of 9 minutes. In addition, a lower SNR (no scan repetitions) modified z-spectrum was acquired similar to the phantom protocol. 51 APT-SAFARI scans (204 images) were acquired with the frequency offset f swept from -450Hz to $+450\text{Hz}$ in steps of 18Hz , thereby simulating B_0 shifts of the water line. Total scan time was just under 14 minutes.

The sensitivity of APT-SAFARI imaging to chemical exchange was characterized by studying the effect of saturation power on the image intensity, similarly to the QUESP technique (39). Power was modulated by changing the delay time between saturation pulses while keeping the total saturation time constant. Due to RF duty cycle limitations on the scanner, the minimum TR_{RF} allowed for a 9ms pulse was 15ms. Therefore, TR_{RF} could only be increased, thereby decreasing power. Seven APT-SAFARI scans were acquired at the amide proton frequency ($f = 0\text{Hz}$) with $\text{TR}_{\text{RF}} = 20, 30, 45, 60, 80, 100 \text{ and } 150\text{ ms}$ corresponding to a CW RF power $B_{\text{CW}} = 0.58, 0.39, 0.26, 0.19, 0.15, 0.12, \text{ and } 0.08\mu\text{T}$, respectively. As TR_{RF} increases from 15ms to 150ms, the number of saturation pulses applied in the 3 second saturation module decreases from 200 to 20, thereby reducing the average saturation power. Each scan was repeated 6 times to increase SNR, resulting in a total acquisition time of 11 minutes.

Image analysis

Image analysis was performed in MATLAB 7.6 (Mathworks, Natick MA). All images were normalized voxel-by-voxel by the unsaturated reference image S_0 . For the high SNR APT-SAFARI scans, quantitative maps of MTR_{asym} and $\text{MTR}_{\text{SAFARI}}$ were calculated voxel-by-voxel according to: [6]

$$MTR_{asym} = S_{sat}(\omega_-)/S_0 - S_{sat}(\omega_+)/S_0 \quad [6]$$

$$MTR_{SAFARI} = S_{sat}(\omega_-)/S_0 + S_{sat}(\omega_+)/S_0 - [S_{sat}(SAFARI)/S_0 + S_{sat}(SAFARI')/S_0] \quad [7]$$

where $\omega_- = 2\pi f - \omega_s$ and $\omega_+ = 2\pi f + \omega_s$. MTR_{asym} and MTR_{SAFARI} were each averaged across the entire slice excluding the skull and ventricles. Results are presented as the mean MTR for each volunteer with standard deviations across the slice. In addition, the error in the APT measurement due to the offset f was calculated voxel-by-voxel by $error = [MTR(f) - MTR(f = 0)]/MTR(f = 0)$, and averaged across all volunteers.

For the lower SNR modified z-spectrum scans, B_0 correction of the single frequency images was performed following a previously published method (8) in order to remove unintentional B_0 inhomogeneity shifts across the FOV before calculating MTR_{asym} . The single frequency images $S_{sat}(\omega_-)$ and $S_{sat}(\omega_+)$ were reordered to generate a standard z-spectrum. The z-spectrum was fit voxel-by-voxel to a 30th order polynomial. The resulting polynomial was evaluated with a frequency resolution of 1Hz. The frequency with the minimum fit intensity was assumed to be the water peak. A map of the water frequency deviation from 0 was generated. To correct for B_0 inhomogeneity across the FOV, the z-spectrum was interpolated by a piecewise cubic spline to a resolution of 1Hz and shifted by the water frequency deviation such that the water resonance occurred at 0Hz for all voxels. After B_0 correction, the z-spectrum was resampled back to its original resolution (30Hz steps for control phantom data, 14Hz steps for BSA phantom data, 18Hz steps for human data). The resulting corrected images were used to calculate MTR_{asym} according to Eq. [6]. MTR_{SAFARI} was calculated from the original data without B_0 correction according to Eq. [7]. The control phantom MTR maps were then averaged for each of the eight solutions across the four tubes with identical relaxivities. The human MTR maps were averaged across the entire brain slice, excluding the skull and ventricles. Results are presented as the mean MTR across all volunteers with standard errors.

For the APT-SAFARI scans as a function of saturation power, the MTR parameters were calculated voxel-by-voxel according to Eqs. [6] and [7] with $f = 0$ Hz. MTR_{asym} and MTR_{SAFARI} were each averaged across the entire slice excluding the skull and ventricles. Results are presented as the mean MTR across all volunteers with standard errors.

Results

The control phantom study confirms that MTR_{SAFARI} does not generate erroneous contrast in the absence of CEST agent. Figure 3 illustrates the effect of frequency shifts on APT imaging in the control phantom without chemical exchange. For clarity, only three of the eight different $MnCl_2$ solutions are shown, including the longest and shortest T_2 solutions. It can be seen that when the off-resonance saturation is applied at the amide proton frequency ($f = 0$ Hz), both MTR_{SAFARI} and MTR_{asym} are zero regardless of T_2 , as expected. Moreover, MTR_{SAFARI} remains below 0.5% for frequency offsets of at least ± 120 Hz for all relaxation times. Increasing T_2 increases the stability of MTR_{SAFARI} , with the longest T_2 solution remaining at $MTR_{SAFARI} < 0.5\%$ for up to ± 180 Hz. Therefore, T_2 does not affect the amplitude of MTR_{SAFARI} as long as water saturation remains small. Instead, it modulates the frequency offset at which direct water saturation becomes significant and can no longer be removed by MTR_{SAFARI} . MTR_{asym} , in contrast, deviates from zero as the frequency offset increases. $|MTR_{asym}|$ exceeds 1% before the smallest offset of ± 30 Hz. It reaches a value greater than 3%, which is on the order of the expected *in-vivo* APT effect, at a

frequency offset of only ± 60 Hz, except for the longest T_2 solution, which remains below 3% up to ± 90 Hz. In addition, the rate of change of MTR_{asym} increases with decreasing T_2 , which induces additional errors in the APT measurement.

A BSA protein phantom was imaged to test the sensitivity of SAFARI to amide and aliphatic proton features and to study the robustness of SAFARI against frequency offsets in the presence of chemical exchange. The standard z-spectrum shown in Figure 4b reveals the amide proton peak at 350 Hz or 2.75 ppm, consistent with previous measurements (31). Even the more selective saturation preparation ($p_w=12$ ms, $TR_{\text{RF}}=20$ ms, data shown in Figure 4) has a fairly broad saturation bandwidth, as can be seen by the width of the water and amide peaks. The water peak has a full width at half maximum of 320 Hz. Therefore, when the RF preparation is applied at the amide proton frequency, these should remain mostly fully saturated even if B_0 offsets shift the line by up to ± 160 Hz. Note, also, that neither of the two RF preparations used produced noticeable saturation transfer from aliphatic protons and no peak was detected in the aliphatic region of the spectrum. Prior work (31) with BSA has shown a more pronounced aliphatic peak when much lower power is applied. Our data cannot exclude some level of aliphatic contribution to the SAFARI signal, however, the major contribution to the MTR_{SAFARI} measurement is expected to be from amide protons alone. The MTR_{SAFARI} spectrum is shown in Figure 4a. On resonance with the amide protons ($f = 0$ Hz), the baseline SAFARI signal is $2.0\% \pm 0.3\%$. In the presence of frequency offsets f , MTR_{SAFARI} remains within 0.5% of baseline in the range of offsets from -140 Hz to 100 Hz. Therefore, the SAFARI strategy can successfully saturate amide protons even when B_0 offsets shift the line and remains insensitive to B_0 shifts of at least 100 Hz. These phantom results are expected to provide a lower bound for the performance of SAFARI *in-vivo*. Since the resonance of *in-vivo* amide protons is centered around 450 Hz instead of 350 Hz, the robustness of SAFARI to B_0 shifts should increase, as will be seen below.

Figure 5 compares a representative high SNR *in-vivo* MTR_{SAFARI} map at $f = 0$ Hz with the corresponding uncorrected MTR_{asym} map and the water frequency map derived from the lower SNR z-spectrum. The water frequency map (Fig. 5a) shows large positive B_0 shifts at the front of the brain, negative B_0 shifts on the left side of the brain and small positive or zero shifts elsewhere. As shown in Figure 3, positive B_0 shifts ($f < 0$) lead to increased MTR_{asym} values, while negative B_0 shifts ($f > 0$) lead to decreased MTR_{asym} values. However, the MTR_{asym} map (Fig. 5b) displays mostly negative values throughout the brain (except at the front of the brain), even in regions with zero or small positive B_0 shifts where MTR_{asym} should be increased, indicating that the contrast is dominated by intrinsic MT asymmetry rather than APT or small B_0 shifts. In comparison, the MTR_{SAFARI} map (Fig. 5c) values are positive suggesting that the proposed APT-SAFARI method can successfully separate the amide proton transfer signal from the MT signal. MTR_{SAFARI} also performs much better in the presence of B_0 inhomogeneity than MTR_{asym} . Comparing Figures 5a and 5b shows that MTR_{asym} closely follows the B_0 inhomogeneity distribution. Elevated MTR_{asym} values are found at the front of the brain and decreased MTR_{asym} values on the left side of the brain, corresponding to susceptibility artifacts near air-tissue interfaces seen on the water frequency map. In contrast, the MTR_{SAFARI} map is homogeneous throughout the brain except for a small gray-white matter contrast. There are no apparent erroneous MTR_{SAFARI} values induced by off-resonance effects indicating that MTR_{SAFARI} is insensitive to the range of B_0 inhomogeneity found in the brain.

The effect of B_0 inhomogeneity was further investigated by acquiring APT-SAFARI scans with asymmetric saturation around the water line. Figure 6 shows quantitative MTR maps for RF frequency offsets $f = 0, 100, 150$ and 200 Hz. In addition, mean values of MTR_{asym} and MTR_{SAFARI} for each volunteer are shown in Table 1. In the presence of a 100 Hz offset simulating a B_0 shift of -100 Hz across the entire field of view, MTR_{asym} decreases on

average by 300% compared to baseline and completely fails at detecting the APT effect. Instead it is dominated by asymmetric direct water saturation yielding large negative MTR_{asym} values. At offsets above 100Hz, the errors in MTR_{asym} increase even more reaching over 700% at $f = 200\text{Hz}$. In contrast, MTR_{SAFARI} performs much better and mean values remain within one standard deviation of the $f = 0\text{Hz}$ MTR_{SAFARI} map for offsets $f = 100\text{Hz}$ and $f = 150\text{Hz}$. An average change of $\sim 155\%$ is seen in the map at $f = 200\text{Hz}$, roughly half the error seen in the MTR_{asym} map at $f = 100\text{Hz}$.

The same trends seen in the high SNR images (Figures 5 and 6) are observed on the *in-vivo* modified z-spectrum illustrated in Figure 7. At $f = 0\text{Hz}$, $MTR_{\text{SAFARI}} = 2.0\% \pm 0.3\%$ is in good agreement with the high SNR measurements in Table 1. $MTR_{\text{asym}} = -3.8\% \pm 0.2\%$, however, is quite different from the values derived from the high SNR maps without B_0 correction, because the large positive MTR_{asym} values due to B_0 inhomogeneity above the sinuses have now been removed. Again, we see that MTR_{SAFARI} is positive and MTR_{asym} is negative. In addition, while MTR_{asym} (Fig. 7b) varies quickly with the RF frequency offset, MTR_{SAFARI} (Fig. 7a) gives a much flatter response up to $f \sim \pm 200\text{Hz}$. The error in MTR_{SAFARI} remains under 50% of baseline for a frequency range $f = -216\text{Hz}$ to $f = 162\text{Hz}$. In contrast, the error in MTR_{asym} is less than 50% only in the range $f = \pm 18\text{Hz}$. At offsets above $\sim \pm 200\text{Hz}$, both parameters increase steeply and peak at $f = \pm 450\text{Hz}$ when the saturation is directly on resonance with the water line (saturation at $+900/0\text{Hz}$ and $0/-900\text{Hz}$).

The power dependence of MTR_{asym} and MTR_{SAFARI} is shown in Figure 8. At the highest power ($TR_{\text{RF}}=15\text{ms}$, $B_{\text{CW}}=0.78\mu\text{T}$), $MTR_{\text{asym}} = -0.3\% \pm 0.3\%$ is negative, as seen previously. Again, the value of MTR_{asym} calculated here without B_0 correction does not match the value calculated from the modified z-spectrum data with B_0 correction, but is in good agreement with the uncorrected high SNR measurements in Table 1. This again, indicates that without B_0 correction, MTR_{asym} fails at quantifying the APT effect. As TR_{RF} increases beyond the chemical exchange time, the APT contrast should decrease due to inefficient amide proton saturation. This was observed experimentally by increasing TR_{RF} up to 150ms. MTR_{asym} initially decreases, then reaches a minimum at -3.4% when $TR_{\text{RF}}=80\text{ms}$ ($B_{\text{CW}}=0.15\mu\text{T}$), and starts increasing. Because MTR_{asym} is the sum of the MT effect (negative) and the APT effect (positive), as TR_{RF} increases and the APT effect decreases, MTR_{asym} becomes more negative. The increase in MTR_{asym} when TR_{RF} exceeds 80ms could be explained by the fact that the MT asymmetry is a function of saturation power (35). As the power decreases, the MT asymmetry lessens, and therefore MTR_{asym} becomes less negative. MTR_{SAFARI} has an initial value of $+2.5\% \pm 0.1\%$ and appears to decrease exponentially towards zero as the power decreases. Note that if amide protons are fully saturated, MTR_{SAFARI} should in principle remain constant as TR_{RF} increases, until the amide proton saturation begins to decrease. Simulations (44) suggest that a minimum CW RF saturation power of $B_{\text{CW}} \approx 0.5\mu\text{T}$ is required to fully saturate amide protons. Therefore, the two shortest TR_{RF} used here should produce full saturation and have similar MTR_{SAFARI} measurements. Although, MTR_{SAFARI} ($TR_{\text{RF}} = 15\text{ms}$) and MTR_{SAFARI} ($TR_{\text{RF}} = 20\text{ms}$) are within one standard deviation of each other no clear plateau was reached at low TR_{RF} , indicating that higher RF power may be needed to fully saturate amide protons. However, the saturation level achieved at $TR_{\text{RF}} = 15\text{ms}$ was sufficient for the APT-SAFARI strategy to be successful as illustrated in the other results. As TR_{RF} becomes larger and amide proton saturation decreases, the assumption that the CEST effect is independent of power no longer holds. Therefore, the CEST effect will tend to cancel out in the MTR_{SAFARI} calculation similar to direct water saturation and MT.

Discussion

We have introduced a novel acquisition technique – saturation with frequency alternating RF irradiation or SAFARI – combined with a new MTR subtraction to measure the amide proton transfer effect *in-vivo*. The SAFARI technique was designed to be insensitive to B_0 inhomogeneity and MT asymmetry. We have shown by comparing uncorrected MTR_{asym} and MTR_{SAFARI} phantom and *in-vivo* results, that MTR_{SAFARI} can reliably measure the APT effect even in the presence of B_0 shifts and MT. Both phantom and human studies indicate that MTR_{SAFARI} can measure the APT signal accurately for frequency offsets up to about $\pm 180\text{Hz}$ at 3T. This frequency range is well above the range of susceptibility offsets that were observed in the brain (see Figure 5a), removing the need for additional B_0 correction of APT images. MTR_{asym} , in contrast, relies on symmetric subtraction around the water line to cancel direct water saturation. This approach is intrinsically dependent on the B_0 homogeneity as was seen in both modified z-spectra data and quantitative brain maps. Unless specialized B_0 correction is used, standard MTR_{asym} images closely reflect the field inhomogeneity rather than the APT effect, as was observed in Figure 5. In addition, even after B_0 correction, MTR_{asym} maps are negative even though the APT effect should yield positive MTR_{asym} values. This is caused by intrinsic asymmetry in the z-spectrum, with the negative offset frequencies having lower intensities than the positive frequencies. This asymmetry is a well-known effect of conventional MT from semisolid protons (33–35) and has been observed in other APT experiments (9, 13, 17, 18, 20, 41). Not only does MTR_{SAFARI} largely remove the need for B_0 correction, it also appears to eliminate MT contamination from the APT maps. Because the APT-SAFARI scan only acquires four images, instead of dozens needed to produce a full z-spectrum for B_0 correction, scan times can be dramatically reduced. Therefore, the SAFARI approach could help make APT, and more generally CEST, a clinically viable research tool.

In this work, we have applied SAFARI imaging to acquire CEST images of amide protons on the backbone of endogenous proteins. In principle, the SAFARI technique is not limited to amide proton exchange. SAFARI could also be used to acquire CEST images of other exchangeable protons as long as the two main assumptions are met. Recall that for SAFARI to be successful, saturation of the water line must be linear with power, i.e. direct water saturation must be small, and the exchangeable protons must be almost fully saturated. These assumptions impose limits on the design of the RF saturation module. The pulse shape, width and repetition rate must be optimized to maximize saturation of exchangeable protons while minimizing direct water saturation. For the case of amide proton exchange, we found that a 9ms Blackman-shape pulse with $TR_{\text{RF}} = 15\text{ms}$ produced a saturation bandwidth broad enough to saturate the amide protons at 3.5ppm even in the presence of B_0 shifts, while within the constraint that the bandwidth not be so broad as to produce strong direct water saturation. Given these parameters, direct water saturation remains insignificant for frequency offsets up to $f = 180\text{ Hz}$, as we've seen from the z-spectra data. At this frequency offset, the off-resonance saturation is applied at a frequency $\nu = \omega_s/2\pi - 180\text{Hz} = 270\text{Hz}$ from the water line. Therefore, the saturation module used here can be applied for SAFARI imaging of exchangeable protons with chemical shifts greater than 270Hz (2.1ppm at 3 Tesla). For protons with smaller chemical shifts, different pulse parameters must be employed to provide a more selective saturation.

The second assumption also imposes a limit on the maximal chemical exchange rate of the protons of interest. If the exchange time is so fast that saturation of the amide protons is not achieved then the SAFARI approach will not be successful. This places requirements on the amplitude and repetition rate of the RF pulse train. For the pulse parameters used here, simulations (42) suggest that SAFARI will only detect exchangeable protons with exchange rates smaller than 200Hz. For faster exchanging protons, higher RF power and potentially

simultaneous dual frequency irradiation with pulsed or continuous wave RF would be possible options to pursue.

The experiments described were optimized for the frequency and expected exchange rates of amide protons, but the possibility of other contributions to the SAFARI signal *in-vivo* cannot be excluded. Nearby amine protons (2) would likely be affected by the saturation pulses we employed, but their typically very high exchange rates would likely ensure saturation was not achieved and therefore the contribution to SAFARI in our experiments should be small. Lines on the other side of water may also contribute to SAFARI. In particular, a number of lines which exchange with water through spin exchange rather than chemical exchange have been reported, for example see (4,7–9,45). A positive contribution of these lines to our SAFARI images, and a negative contribution to asymmetry analysis should be expected.

Conclusion

We have developed a new method for measuring amide proton transfer contrast without errors caused by direct water saturation and magnetization transfer. This feasibility study demonstrates that MTR_{SAFARI} is much more robust in the presence of B_0 inhomogeneity than MTR_{asym} and minimizes the need for specialized B_0 correction. In addition, the confounding MT asymmetry is removed from MTR_{SAFARI} maps, allowing for accurate quantification of the CEST effect. Further evaluation is needed to assess whether APT-SAFARI imaging will permit improved characterization of brain pathology in clinical applications.

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Figure 1.

Schematic illustration of the APT-SAFARI experiment. The spectral line represents the free water signal. W, MT and CEST are the signal loss attributed to direct water saturation, conventional magnetization transfer and amide proton transfer, respectively. The plus and minus subscripts indicate the effects induced by RF saturation applied at the amide proton frequency ($\omega_s = +3.5\text{ppm}$) and the opposite frequency ($-\omega_s$), respectively. For clarity, W is depicted to be symmetric around the water line, while MT is not. Because of the MT asymmetry, the standard two-offset CEST difference $MTR_{asym} = [S_{sat}(-\omega_s) - S_{sat}(+\omega_s)]/S_0$ is not equal to the CEST effect. The SAFARI image with half the saturation power applied simultaneously at the control and label frequencies contains the contributions of W and MT at both frequencies, as well as signal decrease due to APT. Therefore, the difference $MTR_{SAFARI} = [S_{sat}(-\omega_s) + S_{sat}(+\omega_s) - 2S_{sat}(SAFARI)]/S_0$ isolates the CEST effect without contamination from B_0 inhomogeneity and asymmetric MT.

Figure 2.

Pulse sequence for APT-SAFARI imaging with a pulsed off-resonance saturation module followed by a single-slice EPI readout. A 3 second train of Blackman inversion pulses ($\text{pw}=9\text{ms}$, $\text{TR}_{\text{RF}}=15\text{ms}$, flip angle = 180° , average RF power = $0.78\mu\text{T}$) was used. Each scan consists of acquiring four images: a) Standard CEST image with saturation at the labile proton frequency ($\omega_+ = 3.5\text{ppm}$ for amide protons). b) SAFARI image with saturation alternating between the label and control frequencies. c) Standard CEST control image with saturation at the control frequency ($\omega_- = -3.5\text{ppm}$ for amide protons). d) SAFARI image with saturation alternating between the control and label frequencies.

Figure 3.

Modified z-spectra in the control phantom (no CEST agent) demonstrating the effect of RF saturation frequency shifts – similarly to a B_0 offset – on the APT measurements. Results are shown for three $MnCl_2$ solutions. a) MTR_{SAFARI} is consistent with zero and independent of frequency offset and relaxivity up to $\sim \pm 180$ Hz. b) MTR_{asym} varies quickly with frequency offset and relaxivity parameters.

Figure 4.

APT imaging of the BSA phantom. Data plotted for saturation with RF parameters $p_w = 12\text{ms}$ and $TR_{RF} = 20\text{ms}$. a) Mean MTR_{SAFARI} in the BSA phantom as a function of frequency offset f . Error bars represent the standard deviation across the phantom. MTR_{SAFARI} has an nearly constant amplitude for frequency offsets of at least $\pm 100\text{Hz}$. b) The z-spectrum of the BSA phantom shows the amide proton peak at 350Hz and no aliphatic proton features. The vertical line is located at 350Hz . Error bars have been omitted for clarity.

Figure 5.

APT imaging results in a representative human volunteer. a) B_0 map calculated from the low SNR z-spectrum images by the B_0 correction algorithm. b) MTR_{asym} map and c) MTR_{SAFARI} map calculated from the high SNR APT images without B_0 correction. The uncorrected MTR_{asym} map shows large spatial intensity variation over the brain, which corresponds to the water frequency shift map. The uncorrected MTR_{SAFARI} map shows a remarkable improvement in homogeneity over the brain.

Figure 6.

Comparison of MTR_{asy} and MTR_{SAFARI} maps calculated from high SNR APT images as a function of RF frequency offset. The frequency offset f mimics shifts of the water line due to B_0 shifts of $-f$. a) When displayed with the same contrast, MTR_{asy} deviates from baseline becoming more and more negative as f increases, while MTR_{SAFARI} remains invariant. b) The same MTR_{SAFARI} images from (a) displayed with more contrast to better show the quality of the MTR_{SAFARI} images and the deviation with frequency offset.

Figure 7.

APT modified z-spectra averaged across the entire brain slice in four healthy volunteers. Error bars represent the standard error of the mean over the 4 volunteers. a) MTR_{SAFARI} as a function of induced frequency offset shows the APT measurement remains fairly stable up to $f \sim \pm 200$ Hz. At $|f| > 250$ Hz, MTR_{SAFARI} increases steeply as direct water saturation becomes significant. b) MTR_{asym} – calculated after B_0 correction – as a function of induced frequency offset varies quickly with f because it is dominated by direct water saturation. The negative value of MTR_{asym} at $f = 0$ Hz is due to asymmetry in the conventional MT effects.

Figure 8.

APT results in four healthy volunteers as a function of the saturation module RF pulse repetition time. Error bars represent the standard error of the mean over the 4 volunteers. The data point at $TR_{RF} = 15$ ms was derived from the high SNR images in Fig 4 recalculated with only 6 averages to match the SNR of the images acquired as a function of TR_{RF} . a) MTR_{asym} – without B_0 correction – is initially negative due to MT asymmetry. As TR_{RF} increases, MTR_{asym} initially becomes more negative and then reaches a minimum around a TR_{RF} of 80 ms. This reflects amide proton exchange and MT asymmetry effects. b) MTR_{SAFARI} is initially positive and decreases approximately exponentially towards 0 as TR_{RF} increases.

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