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Comparison of T_2 and LASER $T_2^\dagger$ Image Contrast in a Rat Model of Acute Cerebral Ischemia

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Abstract

Previous studies have shown that $T_2^\dagger$ -weighted magnetic resonance images acquired using Localization by Adiabatic Selective Refocusing (LASER) can provide early tissue contrast following ischemia, possibly due to alterations in microscopic susceptibility within the tissue. The purpose of this study was to make a direct *in-vivo* comparison of T_2 -weighted, $T_2^\dagger$ -weighted, and diffusion-weighted image contrast during acute ischemia. Acute middle cerebral artery (MCA) occlusion was attempted in fourteen rats using a modified Tamura approach incorporating electrocoagulation of the left MCA. $T_2^\dagger$ -weighted LASER images (TE = 108 ms), T_2 -weighted CPMG images (TE = 110 ms), and diffusion-weighted images (b-value = 105 s/mm²) were acquired at 4 Tesla within 1.5 hours of ischemia onset. Tissue contrast in the MCA territory was quantified for histologically verified ischemic tissue (N=6) and in sham controls (N=4). $T_2^\dagger$ -weighted LASER images demonstrated greater contrast compared to the T_2 -weighted CPMG images, and more focal contrast compared to the diffusion-weighted images, suggesting different contrast mechanisms were involved.

Keywords

T_2 ; $T_2^\dagger$; LASER; CPMG; diffusion; contrast; ischemia

Introduction

Stroke is the third leading cause of death in North America (1), and it refers to any damage to the brain caused by abnormalities in blood supply, such as the occlusion of a vessel due to a blood clot (embolic stroke) or vessel rupture (hemorrhagic stroke). In the first case, the embolus deprives the affected region of oxygen. Metabolic and structural changes occur, such as lactate production (2), decreased water diffusion (3), alteration of the relative concentration of ²³Na ions (4) and alteration in the relative concentration of oxy- and deoxy-hemoglobin (5). Various magnetic resonance imaging methods has been shown to be sensitive to the above changes and can be used to the assess tissue damage. For example, under certain conditions, the combination of diffusion and perfusion weighted imaging has been shown to identify viable tissue in the early hours of acute ischemia (< 4 hours) (6). However more sensitive imaging biomarkers are

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still required to better define the acute pathophysiological state of the tissue and unequivocally identify tissue that is destined to die.

In the chronic state (> 24 hrs), an increase in tissue water transverse relaxation time (T_2) visualized on T_2 weighted images is clinically accepted as the standard for the detection of tissue death. However, clinically, T_2 contrast is typically absent or small during the acute stage of ischemia. In animal models, the acute changes in T_2 that have been reported have been attributed to changes in water content of the ischemic brain (7), changes in cerebral blood volume (8), oxygen concentration (9) and deoxyhemoglobin levels (5). For example, using a multi-slice Carr-Purcell-Meiboom-Gill (CPMG) sequence and a rat middle cerebral artery occlusion (MCAO) model, Hoehn-Berlage et al. showed significantly elevated T_2 values at 4.7 T after 3 hours of ischemia (3). Rudin et al. (10) also followed the progression of ischemic lesions in a rat model at 4.7 T by diffusion, T_2 and perfusion MRI. Early detection of the lesion was observed with diffusion and MR perfusion imaging and a small lesion was detected in T_2 -weighted images at 1 hour. Calamante et al. (11) observed an early *decrease* in T_2 (minutes after occlusion), followed by a rapid increase and subsequent decrease at 3 hours during permanent middle cerebral artery occlusion in a rat model at 8.5 T while Van Bruggen et al. (12) demonstrated changes in diffusion and T_2 relaxation times in a rat model of focal ischemia, reporting maximally increased T_2 relaxation times at 24 hours following lesion induction in rats. Consistent with these results, Grome et al. (13) and Dietrich et al. (14) showed that water content is at its greatest between 4 and 24 hours following ischemic insult.

Despite these results in animal models, T_2 -weighted imaging in patients with acute stroke is not routinely used to visualize tissue damage. Although diffusion weighted MR imaging may provide early evidence of ischemia, more sensitive imaging techniques are needed to discriminate between viable and non-viable tissue during the acute phase of stroke. Recently, the Localization by Adiabatic Selective Refocusing (LASER) pulse sequence has demonstrated increased sensitivity to water transverse relaxation caused by changes in microscopic susceptibility compared to conventional T_2 weighted imaging (15). Since tissue susceptibility may change following ischemia due to alterations in the relative concentration of oxy- and deoxy- hemoglobin, and cell swelling, sensitivity to this tissue property may provide further insight into the evolution of tissue damage following ischemia. The LASER pulse sequence combines high bandwidth adiabatic full passage (AFP) radio frequency (RF) pulses with slice select gradients to produce sharp excitation profiles with minimal refocusing errors (16) and can be exploited to accurately measure the apparent transverse relaxation time (T_2^+), (17,18) from high resolution, reduced field of view images. The rate of transverse signal decay during LASER is affected by several mechanisms including the diffusion of spins through tissue regions with different magnetic susceptibility (17), and both T_2 and $T_{2\rho}$ relaxation mechanisms (19,20). The contribution of $T_{2\rho}$ relaxation during LASER, which occurs during the AFP pulses when magnetization is locked in the plane perpendicular to the time-dependent effective magnetic field produced by the RF pulse (B_1), has recently been described by Michaeli et al. (19). LASER images have already demonstrated increased signal intensity *in vivo* minutes after the onset of acute embolic ischemia in rabbits (21). The purpose of this study was to make a direct *in-vivo* comparison of image contrast between T_2^+ -weighted LASER, T_2 -weighted CPMG and conventional diffusion-weighted imaging in the acute phase of permanent ischemia. It was hypothesized that the LASER images would show enhanced contrast in ischemic tissue, compared to CPMG and diffusion.

Methods

Animal Model

Fourteen male Sprague-Dawley rats weighing 250-310 g, were studied using a model of focal cerebral ischemia as previously described (22). The study was approved by the University Council of Animal Care at the University of Western Ontario.

Briefly, anesthesia was induced (5% in air/O₂) and maintained (2-2.5% in air/O₂) with isoflurane by way of endotracheal intubation. The Middle Cerebral Artery (MCA) was occluded using a slightly modified version of the Tamura technique (22,23). The animal was placed in a custom built MR compatible frame, and a small T-shaped skin incision was made at the midpoint between the left orbit and the external auditory canal. Subsequent dissection was carried out using microsurgical techniques. The skin incision was retracted and the temporalis muscle was dissected away from the skull along the temporal line. The muscle was then retracted ventrally. A craniotomy was made at the junction between the medial wall and the roof of the infero-temporal fossa. Following the removal of the dura the MCA was occluded by cautery between its cortical branch to the rhinal cortex and the lateral striate arteries. The craniotomy was covered by a small piece of gel foam, the temporalis muscle was sutured with 1-2 stitches, the soft tissue was allowed to fall back into place, and the skin was closed using 5-7 sutures in a simple interrupted pattern. During the surgical procedure the rats were ventilated to normocapnia. Rectal temperature was maintained between 37.0 and 37.5 °C at all times. Following surgical preparation the rats were transported to the MRI facility for imaging during which heart rate and oxygen saturation were monitored using an MRI compatible pulse oximeter.

MR Imaging

Experiments were performed using a 4 Tesla whole body MR scanner, consisting of an Oxford Magnet Technology (Oxford, UK) 90 cm magnet, Varian (Palo Alto, CA) Unity INOVA console, and Siemens (Erlangen, Germany) Sonata gradient system. The rat was positioned in a plexiglass holder, which was secured to the MR patient table. A proton birdcage radio frequency (RF) coil (9 cm diameter) was inserted over the rat's head. Manual shimming (using linear and Z² shims) was performed to maximize the magnetic field homogeneity within the brain. Following shimming, T₁-weighted inversion prepared turbo FLASH images (FOV=8 × 8 × 4 cm³, TR/TE/TI = 15/7.6/500 ms, 256 × 256 × 8 acquisition matrix) were acquired to correlate histologically identified ischemic tissue with in-vivo anatomical features. LASER (16) images of the ischemic area were then acquired. The LASER pulse sequence (17) begins with a non-selective adiabatic half passage (AHP) pulse followed by six adiabatic full passage (AFP) slice selective HS2-R15 (24) pulses for volume selection. The HS2-R15 pulses are amplitude and phase modulated hyperbolic secant pulses, phase cycled according to the Malcolm-Levitt phasing scheme (MLEVn) (26) with pulse bandwidth (A) = 18,850 rad/s and pulse length (T) = 0.0025s. The nomenclature used to describe these AFP pulses is HS2-R15, where R15 describes the adiabaticity factor, R=AT/π=15. Crusher gradients of alternating sign and varying amplitude were used on each axis to dephase flow effects and eliminate stimulated echoes and signals outside of the region of interest. A four shot 3D EPI readout was appended to the original LASER spectroscopy localization pulse sequence (TR/TE = 4000/30 ms, 2 × 3 × 0.5 cm³ LASER voxel excitation, 4 × 4 × 4 cm³ EPI FOV, 128 × 128 × 8 acquisition matrix, total acquisition time 4 min 50 sec) to obtain a reduced field of view image of the rat brain and assess the effectiveness of the LASER localization. The multi-shot EPI readout reduced the T₂*-weighting in the images, which can also generate signal changes during ischemia (25). T₂[†]-weighted images were obtained at an echo time of 110 ms by inserting 16 nonselective HS1-R10 (24) AFP pulses (Carr-Purcell (CP) pulse train) prior to the LASER localization while maintaining a constant refocusing time (2τ_{CP}, (17)). The AFP pulses were phase cycled

according to the MLEVn (26) scheme with pulse bandwidth (A) = 12.566 rad/s and pulse length (T) = 0.0025 s. The nomenclature used to describe the AFP pulse is HS1-R10, where R10 describes the adiabaticity factor, $R = AT/\pi = 10$. In the LASER portion of the sequence A was increased to 18,850 rad/s to minimize voxel displacement. T_2 -weighted images were obtained with a 2D CPMG pulse sequence that produced 22 echoes with TE ranging from 6 ms to 122.6 ms (3000 TR, 4×4 FOV, 128×64 acquisition matrix, total acquisition time 3 min 15 seconds). The slice thickness, field of view and time between refocusing pulses were matched to the corresponding LASER parameters. For image and contrast comparison the closest echo time of the series (TE = 108 ms, 17th echo) was selected to compare to the LASER images acquired at an echo time of 110 ms. Diffusion weighted images were acquired by varying the pulsed gradients (G_{diff}) in a conventional pulsed gradient spin echo (PGSE) sequence while keeping the diffusion time (Δ) and the pulsed gradient width (δ) constant (TR/TE = 2000/60 ms, Δ = 40 ms, δ = 10 ms, G_{diff} = 0.4 G/cm, b -values in the range of 4 to 269 (only 105 s/mm² was used in data analysis)).

Following the initiation of ischemia the rat was positioned in the magnet centre and LASER images were acquired within 40-60 minutes. Immediately after the LASER images, a set of CPMG and diffusion images were acquired 60-90 minutes following initial ischemia onset. The order of acquiring images was always the same after the onset of ischemia. A second set of LASER and CPMG images were obtained 2.5-3.0 hours following ischemia onset in a subset of animals to determine whether the infarct became visible on the T_2 -weighted images. Rats were maintained on anesthetic and then euthanized with an overdose of pentobarbital four hours after ischemia onset. The brain was then extracted and prepared for histological analysis.

Histology

Rat brains were removed and sliced into 2 mm coronal sections matched as closely as possible to the orientation of the LASER images. Tissue sections were incubated with 2% 2,3,5-triphenyltetrazolium chloride (TTC) for 30 minutes to identify viable and non-viable tissue (27,28) and then 10% buffered formalin. TTC stains viable tissue red, while tissue without viable mitochondria remains unstained (white, shown in Fig.1). TTC stained sections were scanned with an EPSON Perfection 636U flatbed scanner (EPSON, Canada Ltd., Figure 1A) and compared to the LASER, CPMG and diffusion images.

Data Analysis

Signal intensity (SI) changes from LASER, CPMG and diffusion-weighted images were analyzed in a similar manner: a region of interest (ROI) based on histology was selected in the LASER images to encompass the stroke region (region of elevated signal intensity, Fig. 1) in the MCA territory (regions outlining the stroke contained 35 pixels on average). The same ROI was then used to measure SI in the CPMG and diffusion images, and was flipped about the midline to measure contralateral SI. A similar, small ROI positioned within the MCA territory was used to measure SI from ipsilateral and contralateral regions in animals where no histological evidence of ischemic damage was evident (sham controls). The image processing software Stimulate V5 (Center for Magnetic Resonance Research, University of Minnesota Medical School, Minneapolis, MN) was used for ROI selection and SI calculation. Raw signal intensities were used in the data analysis. Average contrast was calculated for stroke and control groups, defined as $(SI(\text{ipsilateral}) - SI(\text{contralateral})) / SI(\text{contralateral})$. A two tailed t -test was used to compare SI and contrast between groups.

Results

In this study of 14 rats, six showed histological (TTC stain) evidence of infarction, four showed severe damage to the MCA with hemorrhaging in the tissue beneath the cautery location and

were therefore excluded from the study, and 4 showed no evidence of ischemia and were used as sham controls. Animal temperature was monitored and remained stable throughout the study between 37-37.5 °C. Figure 1 shows a typical TTC stained histology image (Fig. 1A) and the corresponding LASER (Fig. 1B, TE = 110 ms), CPMG (Fig. 1C, 108 ms) and diffusion-weighted images (Fig. 1D, $b=105 \text{ s/mm}^2$) acquired between 40-90 minutes. The infarct appears as a white region on the histology image, and is visible in the LASER images (Fig. 1B, acquired 40 minutes post ischemia). Some signal increase was also visible on the diffusion-weighted MR images (Fig. 1D). Comparison of Figures 1B, C, and D demonstrate that greater contrast is observed with LASER and diffusion imaging compared to CPMG. The more focal nature of the increased signal intensity in the LASER (Fig. 1B) images compared to the diffusion-weighted (Fig. 1D) images also suggests sensitivity to different stages of tissue damage.

Signal intensity (SI) in ipsilateral (squares) and contralateral (triangles) regions of the rat brain in the control animals are shown in Figure 2, for LASER (Fig. 2A), CPMG (Fig. 2B) and diffusion-weighted (Fig. 2C) images. No significant differences were noted between the signal intensities in the two hemispheres in all cases. The SI for contralateral (triangles) and ischemic tissue (squares) in all six animals in which ischemia was achieved is shown in Figure 3 for LASER (Fig. 3A), CPMG (Fig. 3B), diffusion (Fig. 3C). Error bars in Figures 2 and 3 represent the standard deviation of pixels within the ROI. The average contrast for LASER, CPMG and diffusion images is shown in Figure 4. Gray areas represent average stroke contrast ($N=6$), and black areas represent average control contrast ($N=4$). Asterisks identify significant differences, $p<0.035$. Figures 3 and 4 demonstrate that significant SI differences were observed between contralateral and ipsilateral hemisphere during the first hour of ischemia with LASER ($p=0.035$, Fig. 4) and diffusion ($p=0.030$, Fig. 4), while these differences are not statistically significant in the case of CPMG ($p=0.16$, Fig. 4).

Discussion

In this study, T_2^+ -weighted LASER image contrast was directly compared to T_2 -weighted CPMG and diffusion-weighted contrast for the detection of tissue damage during the initial 90 minutes of acute ischemia in rats. A small ROI (based on the lesion size at 4 hours on TTC stained histology) was used to measure image contrast. Regions of interest were selected to encompass the stroke region in the LASER images, and were replicated for the data analysis of the CPMG and diffusion images. In that way a more accurate SI comparison was made between image types. Greater T_2^+ -weighted LASER image contrast was observed compared to that seen on T_2 -weighted CPMG images.

As has previously been shown, diffusion-weighted imaging (DWI) was shown to be a sensitive tool for the detection of early ischemic tissue changes (< 30 minutes, (29)). DWI is sensitive to areas of ischemic brain with restricted water diffusion and can detect very small areas of ischemic damage (10,11,30). Diffusion weighted images are frequently used in animal models for early detection of acute ischemia (< 3 hrs) when intervention and reversibility of ischemic injury is possible. A diffusion b -value of 105 s/mm^2 was used in this study, which is low compared to that used clinically for diffusion MRI (usually of the order of 1400 s/mm^2 (29-32)). In this study, even though higher b -values were obtained and showed better contrast in diffusion weighted images, motion artifacts precluded the use of these images and hence a lower b -value was used (105 s/mm^2) for comparison. The ischemic lesion was clearly visible even with a b -value as low as 105 s/mm^2 , but significantly higher contrast would be expected in Fig. 4D if higher b -values were used.

Due to the nature of the contrast, T_2 weighted imaging sequences do not normally show ischemic damage until about 3 hours after occlusion (3). Consistent with these results, Grome et. al. (13) and Dietrich et. al. (14) showed that water content is at its greatest between 4 and

24 hours following ischemic insult. However, despite these results, T_2 -weighted imaging in patients with acute stroke is not routinely used to visualize tissue damage. T_2^+ -weighted LASER images have been shown to be more sensitive than T_2 -weighted images to microscopic susceptibility (19). In the current study, ischemic damage was detected on LASER images within 60 minutes of the start of ischemia. However, LASER images generally demonstrated more localized damage while diffusion images showed more diffuse regions of injury (see Fig. 1). Similar findings of more localized damage in T_2 -weighted images compared to diffusion-weighted images have been reported by Latour et al. (33), Bardtski et al. (34,35), Rudin et al. (10) and Moseley et al. (36). In a rat model of MCAO by electrocoagulation at 4.7 T, Rudin et al. observed lesion size mismatch in diffusion-weighted and T_2 -weighted images at 1 hour, where lesion size was much larger on diffusion-weighted images compared to the T_2 -weighted images. The early diffusion changes agreed well with the final histology performed 9 days after occlusion, while the lesion size on the T_2 -weighted images increased until it reached the size indicated in the diffusion images (9 days after occlusion). These discrepancies were attributed to sensitivity to different aspects of tissue damage. Diffusion weighted imaging is sensitive to free water diffusion in the brain and intracellular water accumulation (cytotoxic edema), which is one of the main changes associated with early ischemia (minutes after occlusion) (10). On the other hand, increased T_2 reflects an increase of extracellular water (vasogenic edema) (10), and hence a later stage of final tissue necrosis. In the current study, the histology was performed at 4 hours, and the extent of tissue damage agreed with the LASER image hyperintensity, not with the changes observed on the diffusion-weighted images. This mismatch likely indicates that the ischemic damage was still progressing. If cell damage had been left to progress to a later time, the lesion on histology would most likely have agreed with that observed in early diffusion-weighted images. This result also demonstrates the potential significance of the LASER contrast, which may be more sensitive to early tissue death, compared to that of diffusion and T_2 . The increased contrast is likely due to greater sensitivity to endogenous microscopic susceptibility gradients, which are reduced due to water accumulation, severe cell loss, and cell swelling in ischemic tissue (21). Kavec et al. (37) has also shown that there is close agreement between the time course of dynamic dephasing (LASER) contrast in ischemia and water accumulation revealed by overall T_2 change and spin density ratio. Since LASER contrast is the result of several mechanisms (including T_2 , $T_{2\rho}$, and diffusion), cytotoxic edema, reduced extracellular volume, lower bulk diffusion and intracellular acidosis may all influence the apparent T_2 (T_2^+) measured by LASER in the early moments of ischemia (37).

There are several factors that could result in the observed difference between T_2^+ and T_2 contrast. In a previous study of phantoms containing microscopic magnetic susceptibility variations (15), a significant difference between T_2^+ and T_2 time constants was shown when the time between refocusing pulses ($2\tau_{cp}$), was small. These differences were attributed to $T_{2\rho}$ mechanisms (dynamic averaging effects such as diffusion or exchange of spins). In these experiments, the T_2 time constants were greater than T_2^+ . These differences were due to the variation in magnetic susceptibility of the medium. The utility of this conclusion for stroke imaging was initially tested in an in-vivo rabbit model of ischemia (21) and further in the current study. In the rabbit model, it was found that contrast within the ischemic lesion was observed much earlier in LASER than in CPMG, and it was hypothesized that this effect was due to *reduced* susceptibility variation in regions of tissue affected by stroke. Water exchange and cell loss are the most notable consequences of ischemic damage. Water accumulation leads to a loss of cells in the areas of damage. Water accumulation is known to increase T_2 , but also leads to a reduction in the endogenous microscopic susceptibility gradients in ischemic tissue, which would account for the sensitivity of the T_2^+ weighted LASER sequence at early time points. Blood deoxygenation is unlikely to cause the effect observed, since deoxyhemoglobin is paramagnetic and as such has an increased volumetric susceptibility which would result in reduction of the signal intensity, while the contrary is observed in this work.

Different methods for inducing MCAO exist in literature. In the current study, a modified Tamura approach was selected, which incorporated craniotomy and cautery of the vessel. The model however can cause severe damage to brain tissue in addition to cauterizing the vessel. Subsequent blood accumulation and hemorrhaging may lead to significant image artifacts. In the current study, four animals had severe tissue damage and hemorrhaging of the studied slice and were therefore excluded. In these animals, a combination of increased and decreased SI was observed in LASER and the tissue later disintegrated after TTC staining due to significant tissue damage during the surgery. Although the results of this study indicated that LASER imaging produced significant contrast in acute ischemia, future work must incorporate different ischemia models with less severe surgical induction of MCAO.

Several limitations must also be acknowledged. The physiological measurement of $p\text{CO}_2$ was not performed in this study. Although $p\text{CO}_2$ levels are important in the evaluation of tissue damage during ischemia, it is unlikely that variations in $p\text{CO}_2$ levels can explain the differences in contrast observed in the current study. In the current study, the location of the ischemic lesion was verified by TTC stained histology. However, registration of MR images with histology was not performed due to difficulties in matching the slice thickness and orientation of the histology and MR images. Future studies will register such histology with the corresponding in-vivo MR image to directly correlate in-vivo signal changes with histological markers. Finally, the low b-values used for diffusion-weighted images limited the observed contrast in these images and may have resulted in some perfusion weighting. Despite these limitations, the early appearance of the ischemic lesion in LASER images holds promise that the combined T_2 , $T_2\rho$, and molecular diffusion contrast obtained with LASER may provide new insight into specific pathological processes during stroke.

Conclusion

In the current study, T_2^+ -weighted LASER, T_2 -weighted CPMG and diffusion weighted MR image contrast were directly compared during acute focal ischemia to evaluate their effectiveness for the early detection of stroke tissue damage. Greater contrast was observed in LASER images compared to CPMG images within minutes of ischemia onset in histologically confirmed ischemic tissue. The greater sensitivity of LASER was attributed to the known sensitivity of LASER to changes in microscopic susceptibility. The LASER pulse sequence has therefore demonstrated the ability to detect acute signal changes due to ischemia and may complement other MR imaging techniques like diffusion and perfusion-weighted imaging in the evaluation of tissue damage due to stroke.

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Fig.1. Representative images from a single study including A) TTC histology slice, LASER B), CPMG C) and diffusion weighted (D) images. The stroke region is outlined by a red circle. The circled region in the TTC section represents damaged tissue while the pink represents viable tissue.

Fig. 2. Signal intensity from regions of interest in ipsilateral (squares) and contralateral (triangles) regions of the brain, obtained with LASER (A), CPMG (B) and Diffusion (C) in control rats. Error bars represent the standard deviation of pixel intensity within the ROI.

Fig. 3. Effect of ischemia on signal intensity (SI) from ROIs in ipsilateral (squares) and contralateral (triangles) areas of ischemic rat brain, obtained with LASER (A), CPMG (B) and diffusion (C). Ipsilateral tissue corresponds to ischemic tissue. Errors represent the standard deviation of pixel intensity within the ROI.

Fig. 4.

Average contrast in LASER (1), CPMG (2) and Diffusion weighted (3) images. Contrast is defined as $(SI \text{ (ipsilateral)} - SI \text{ (contralateral)}) / SI \text{ Contralateral}$ in stroke (gray, N=6) and control (black, N= 4). Asterisks denote significance ($p < 0.035$). All data in this plot was acquired within 90 minutes of ischemia onset. Error bars represent the standard deviation.