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MRI measurement of blood–brain barrier transport with a rapid acquisition refocused echo (RARE) method

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ABSTRACT

Dynamic Contrast Enhanced (DCE) MRI is increasingly being used to assess changes in capillary permeability. Most quantitative techniques used to measure capillary permeability are based on the Fick equation that requires measurement of signal reflecting both plasma and tissue concentrations of the solute being tested. To date, most Magnetic Resonance Imaging (MRI) methods for acquiring appropriate data quickly rely on gradient recalled echo (GRE) type acquisitions, which work well in clinical low field settings. However, acquiring this type of data on high field small animal preclinical MRIs is problematic due to geometrical distortions from susceptibility mismatch. This problem can be exacerbated when using small animal models to measure blood brain barrier (BBB) permeability, where precise sampling from the superior sagittal sinus (SSS) is commonly used to determine the plasma concentration of the contrast agent. Here we present results demonstrating that a standard saturation recovery rapid acquisition refocused echo (RARE) method is capable of acquiring T1 maps with good spatial and temporal resolution for Patlak analysis (Patlak, 1983) to assess changes in BBB Gd-DTPA permeability following middle cerebral artery occlusion with reperfusion in the rat. This method limits known problems with magnetic susceptibility mismatch and may thus allow greater accuracy in BBB permeability measurement in small animals.

1. Introduction

Changes in blood brain barrier permeability are often characterized using Dynamic Contrast Enhanced (DCE) imaging, specifically, by injecting a bolus of contrast agent such as Gd-DTPA intravenously and following changes in tissue and plasma spin lattice relaxation rate, R1 (=1/T1) over time. Under these conditions, changes in R1 (ΔR1) are commonly treated as linear with changes in [Gd-DTPA] [1]. Measurement of R1 vs. time and space yields the spatial distribution of [Gd-DTPA] over time. This permits examination of the dynamics of the exchange of Gd-DTPA across the blood brain barrier that can be modeled with the Fick equation for diffusional flux and analyzed using a Patlak plot [2–4].

Critical to this analysis is measurement of T1 with maximum resolution in time and space. Typically this is done via a Look-Locker method [5,6] with TOMROP [7] and echo planar modifications [8] that work quite well on humans at clinical fields. However, gradient echo type sequences have drawbacks at high fields due to well-documented distortion from magnetic susceptibility mismatch at tissue boundaries and air tissue interfaces [9,10]. These known drawbacks are exacerbated in small animals. Susceptibility mismatch effects also increase with field and preclinical small animal MRI systems are now being delivered at 17.6 T. Moreover, TOMROP uses only a fraction of the magnetization for acquiring each image in the T1 map.

Rapid acquisition refocused echo (RARE) [11] imaging is well established to be relatively insensitive to susceptibility mismatch. Thus it produces good images at high field [10] without spending extensive time shimming. Furthermore, because it is refocused, all the available magnetization is used for image acquisition. But the trade off is that there are many RF pulses potentially heating the animal and the echo times are longer, sometimes leading to a loss of signal due to T2 relaxation. RARE has recently been used to better

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establish baseline values of T1 and T2 at 17.6 T in mice [12]. Thus, we examined RARE with a variable relaxation delay for the measurement of T1 in small animals at high field with the purpose of obtaining data with adequate S/N and resolution for DCE-MRI measurements suitable for Patlak analysis.

2. Materials and methods

This study was conducted in accordance with the Animal Use and Care Guidelines issued by the National Institutes of Health using a protocol approved by the Animal Use and Care Committee at University of California Davis (IACUC protocol #15946). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. The experiments described were conducted using Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) weighing 180–250 g. Animals were anesthetized with i.p. injection of sodium pentobarbital (60 mg/kg body weight) and surgical plane anesthesia was maintained with a quarter dose (15 mg/kg body weight) every 30–45 min. Body temperature was maintained using an electric heating pad during surgery and a water heating pad (Gaymar Inc., Orchard Park, NY, USA) during MRI data acquisition. The left femoral vein was cannulated with PE-10 polyethylene tubing for infusion of gadopentetate dimeglumine (Gd-DTPA - Magnevist; Bayer Healthcare Pharmaceuticals, Wayne, NJ, USA). Animals used for the Patlak experiments were prepared using the middle cerebral artery occlusion (MCAO) model as previously described [13] but reperfused for 18 h after 3 h occlusion as modified from Nagaraja et al. [3].

The RARE experiment was performed on a Bruker Biospec 7T imaging system using ParaVision Version 4 with a modified RARE-VTR method. The imaging parameters were: TE = 10.26 ms, TR array = 168.4, 500.2, 927.1, 1525.9, 2538 and 7500 ms, RARE factor = 16, slice thickness = 2 mm, FOV = 64 × 64 mm, matrix = 128 x 128, and total experiment time = 105 s for each T1 map. MRI data were collected in a saturation recovery mode where TR was varied to collect a series of T1 weighted images from which a T1 map was generated. The measured T1 values for the rat brain were 1772 ± 227 and 2138 ± 384 ms (mean ± SD) for the right (un-occluded) and left (occluded) hemispheres, respectively. This agrees well with previous studies using a similar MCAO with reperfusion model [14]. A representative T1 map is shown as an inset to Fig. 2. Such maps are converted to R1 maps and delta R1 vs. time was calculated. Rectal temperature was monitored with a type T thermocouple to assess possible heating due to SAR (Specific Absorption Rate). During the experiment core temperatures nominally increased at a rate of 0.05 °F/min independent of RARE imaging and did not exceed the normal rat body temperature range.

The data were analyzed using the Patlak Model [2–4] with locally written Matlab code. Briefly, transport is modeled using the equation $C_t(t) = Ki\int C_p(\tau)d\tau + C_p(t)V_p$ where C_t is the Gd-DTPA tissue concentration (per volume), and C_p is the Gd-DTPA arterial plasma concentration (per volume). K_i is the product of the permeability and the surface area per volume (per minute), i.e. the transfer constant for contrast agent movement from the blood to the tissue. V_p = fractional "volume of the various tissue compartments in which water with intravascular-Gd-changed relaxation rates distribute" [1] (ml/g) prior to Gd-DTPA crossing the BBB. In the fast exchange limit $R1 = R1_0 + R1^*[\text{Gd-DTPA}]$ for both tissue and plasma. In general, ΔR1 is the change in pixel R1 following Gd-DTPA infusion. Thus in tissue, $\Delta R1_t(t)$ can be substituted for $C_t(t)$. When Hct = the fraction of blood that is cells, $\Delta R1_p(t)/(1\text{-Hct})$ in the superior sagittal sinus (SSS) can be substituted for $C_p(t)$. Plotting $(1\text{-Hct})C_t(t)/C_p(t)$ vs. "stretch time" = $\int C_p(\tau)d\tau/C_p(t)$, the data can be fit linearly with slope Ki [1].

3. Results

Results showing R1 for the SSS and representative pixels from both hemispheres after MCAO/reperfusion are shown in Fig. 1 along with the Patlak plot for the same data.

Further analysis gave Ki values of 0.00012 ± 0.00085 and 0.00177 ± 0.00254 min^{-1} (mean ± SD) for the right (un-occluded) hemisphere and the left (occluded) hemisphere respectively. These values compare well with those previously published for a similar rat model [3]. The Ki distributions of the left and right hemispheres are plotted in Fig. 2 and mapped in the insert. These results compare well with those published by others [3,15].

4. Discussion

The criteria for obtaining DCE-MRI data useful for characterizing changes in capillary permeability using Patlak analysis are, 1) acquisition intervals short enough that repeated acquisitions capture the dynamics of the process, 2) sufficient spatial resolution to sample precisely from the structures of interest, and 3) sufficient signal to noise to measure changes in contrast agent concentration, both in the tissue and within the vascular space. Given common limitations in MRI imaging, criterion 1 is in conflict with criteria 2 and 3. Criterion 2 is also in conflict with 3 and all of these criteria present a challenge in small animals because almost all structures one wishes to sample are diminished in size.

Most researchers address issues of acquisition time using Look-Locker [5,6] type sequences such as TOMROP [7] or IR/SE EPI [8], but these are known to have limited spatial resolution especially where magnetic susceptibility mismatch can occur [16]. TOMROP is fast due to using a low tip angle (and thus only some of the magnetization) and acquiring a line of k-space for several delays after an inversion. EPI acquires several lines of k-space for each inversion thus increasing acquisition speed. RARE also acquires multiple lines of k-space in a single shot, but uses all of the magnetization. The gradient echo type sequences (TOMROP, IR/SE EPI) also use inversion recovery (IR) to measure T1. Here we use saturation recovery rather than IR to speed up the process. Time is saved with saturation recovery over IR because 5T1's are not required between each scan. Saturation recovery sacrifices some dynamic range in the T1 measurement, but with sufficient S/N this is an acceptable trade-off.

Using higher field magnets can resolve some of these issues because higher field increases signal to noise thus allowing higher spatial resolution and/or shorter acquisitions times. Currently preclinical imaging is commercially available at 17.6 T. However conducting experiments at higher field also leads to image distortion such as those due to magnetic susceptibility mismatch and thus loss of signal from intravoxel dephasing [10]. Moreover, these effects become worse as the field increases. RARE has the advantage that signal is not lost due to intravoxel dephasing. Moreover, the geometric distortions associated with shimming and tissue interfaces in gradient echo type sequences are in large part corrected for in RARE. In some small animal experiments this is a potential advantage because time spent shimming should be diminished.

The issue of spatial distortion is likely to be troublesome when attempting to assess changes in contrast agent concentration within vessels and particularly in the SSS in small animals where a number of tissue borders including bone and air are in close proximity. Some investigators have attempted to limit problems with MRI measurement of plasma contrast agent concentration by using literature values for the arterial input function [17,18] but this

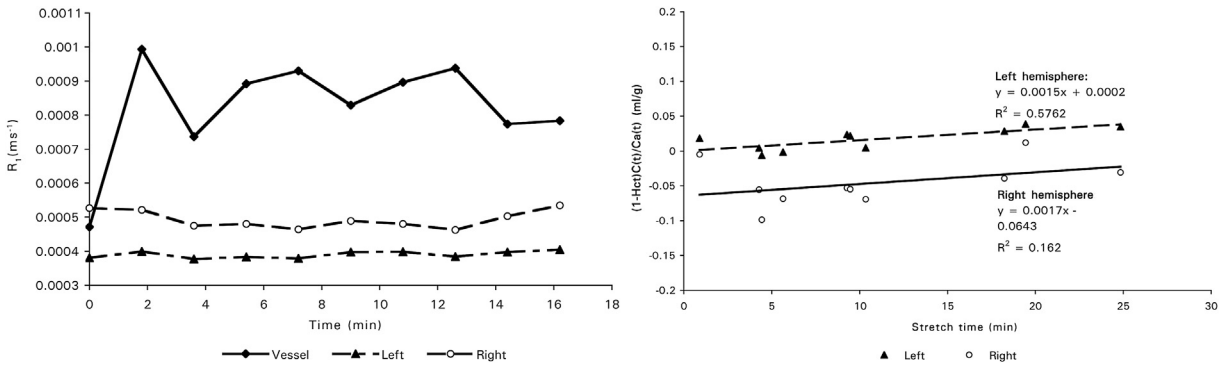

Fig. 1. The graph on the left shows R1 values for 3 pixels before and after Gd-DTPA injection. Closed diamonds correspond to data acquired from the superior sagittal sinus (SSS); the other two sets of data are from symmetrically sampled pixels in the occluded (left) and un-occluded (right) hemispheres. The figure on the right shows the Patlak plot for the same two tissue pixels. Note the F-test shows the data from the un-occluded right hemisphere has a slope = Ki not significantly different from zero, while the slope for the data from the occluded left hemisphere is significantly greater than zero.

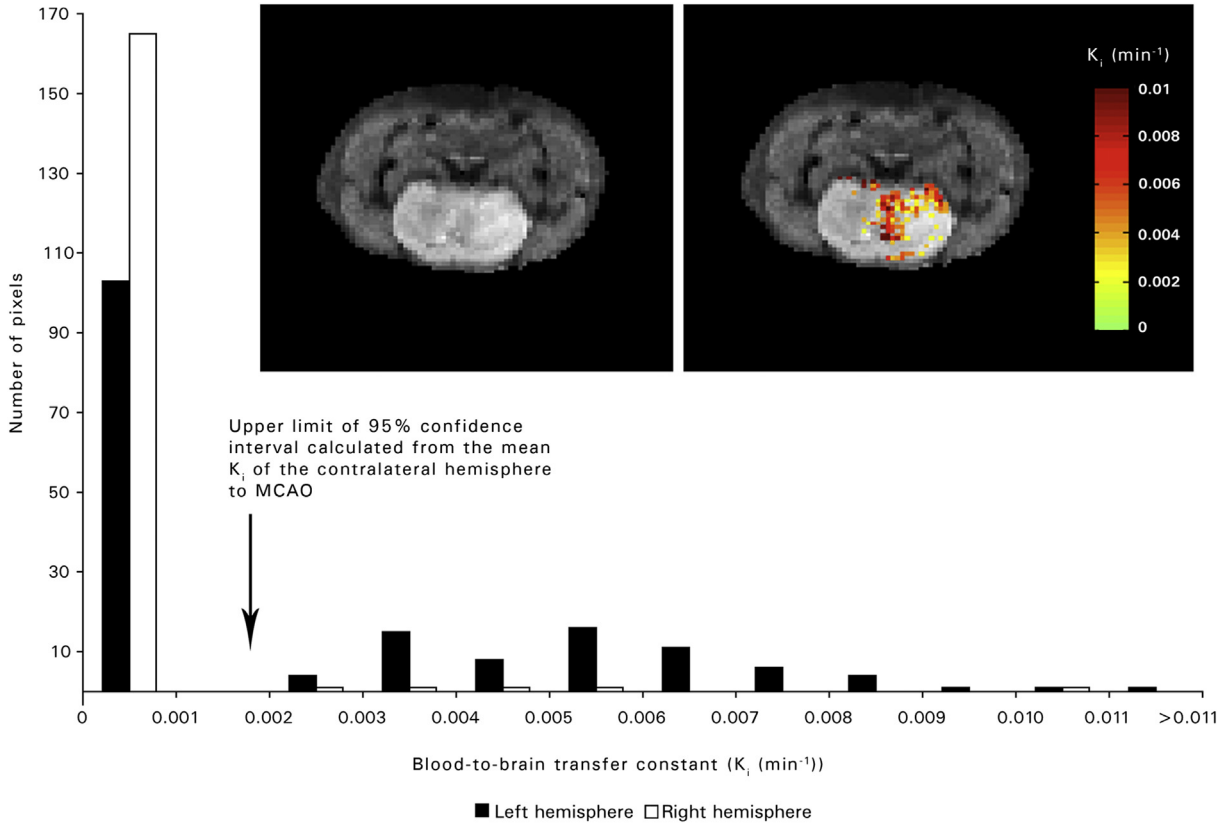

Fig. 2. The K_i distribution. The histogram shows the number of pixels vs. measured K_i has a distribution similar to normal brain [15] but with a subpopulation of pixels corresponding to the ischemia/reperfused region. The left inset shows the T1 map for this data set generated in ParaVision by fitting to a single exponential and using the M_∞ map to segment and mask the noise outside the head. The right inset shows the map of K_i values superimposed on the T1 map. The color table has its max set at 0.01 min^{-1}. The K_i overlay includes all brain K_i values greater than the mean+2SD measured for the un-occluded hemisphere. The 95% confidence limit was calculated using the mean Ki and standard deviation (0.00012 ± 0.00085) from the un-occluded hemisphere yielding an upper 95% confidence limit of 0.00178. Note that the right hemisphere is on the left side of the images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is likely to introduce a variety of errors unless contrast agent injection and intravascular mixing are identical in all experiments. RARE is a fast imaging protocol collecting multiple lines of k–space for each acquisition. In general the image resolution is much better than necessary to adequately sample the brain tissue in which one desires to measure the transfer of Gd-DTPA. However, of equal or greater importance, is resolving the blood vessel that is used to measure the arterial input function (AIF). We use the superior sagittal sinus (SSS) as it has been previously shown to provide a satisfactory sample of the AIF, especially for relatively low permeability vessels such as those of the blood brain barrier [1]. Ideally the acquired images should have one pixel entirely within the SSS. Assuming square pixels (our case) and the SSS is oriented perpendicular to the imaging plane, based upon geometrical considerations, $a \leq d/(2(2\hat{}0.5))$ where d = SSS diameter and a = the resolution, would guarantee at least one pixel entirely within the

SSS. Recent optical [19] and CT [20] studies have measured the SSS diameter to be ~700–750 μm. Our resolution of 500 μm is larger than optimum, but the criteria can be relaxed. If the voxel of interest has a majority of spins from the SSS, since the R1 of the SSS is the largest rate in the voxel, i.e. larger than R1 from non-SSS tissue within the voxel, the R1 of the SSS will dominate the measurement. Again using geometrical arguments, assuming square pixels with side a, and a circular SSS with radius r, the minimum overlap between the SSS and a pixel is when the intersection of four pixels is at the center of the SSS. Thus the criteria for a pixel to have part of the SSS occupy more than 50% of the pixel is $(\pi/4)r^2 > a^2/2$ and with a = 500 micron and r ~ 350 micron, we are very close to fulfilling this condition.

We also note that in principle we have the flexibility to measure the arterial input function (AIF) in the SSS in a slice different than the imaging slice, thus picking out the SSS at it largest dimension. This would naturally fall out in multislice acquisition.

Enhancement of spatial resolution has been addressed recently using a radial keyhole sampling strategy [18]. It remains to be seen whether this technique will gain acceptance, but we note that these investigators also used literature values for the AIF function rather than using their own measurement.

Another advantage of the gradient echo type sequences over RARE is that many fewer pulses are used. Thus the issue of animal heating due to SAR is generally not considered in gradient echo type sequences. However, RARE has many pulses drastically increasing SAR. Here we observed no heating, however these were single slice experiments so this should be carefully tested in multislice experiments.

Most methods used to measure BBB permeability in rodents have been ex vivo and involve measurement of a labeled molecule in the brain after a period of perfusion with blood. These techniques have been used to measure catastrophic brain injury, such as that occurring after MCAO. The technique describe herein will enable the investigator to serially measure subtle changes in BBB permeability over time. This approach will enable evaluation of chronic diseases, such as Alzheimer's disease and vascular dementia, where disease progression is incremental rather than catastrophic.

In conclusion, although current methods for DCE-MRI measurement of capillary permeability are satisfactory for clinical use with relatively large subjects at low field strength, preclinical imaging is trending toward using DCE-MRI in small animals at high field strength for basic science research. Here we have demonstrated that a RARE pulse sequence, previously demonstrated to limit errors due to magnetic susceptibility mismatch to improve resolution, combined with a saturation recovery measurement of T1, previously demonstrated to improve sensitivity, can be used successfully for measuring blood brain barrier permeability at high field in small animals.

Conflict of interest

The authors declare no conflict of interest.

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