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Multiparametric Magnetic Resonance Imaging and Repeated Measurements of Blood-Brain Barrier Permeability to Contrast Agents

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Summary

Breakdown of the blood-brain barrier (BBB) is present in several neurological disorders such as stroke, brain tumors, and multiple sclerosis. Non-invasive evaluation of BBB breakdown is important for monitoring disease progression and evaluating therapeutic efficacy in such disorders. One of the few techniques available for non-invasively and repeatedly localizing and quantifying BBB damage is magnetic resonance imaging (MRI). This usually involves the intravenous administration of a gadolinium-containing MR contrast agent such as Gd-DTPA, followed by dynamic contrast-enhanced MR imaging (DCE-MRI) of brain and blood, and analysis of the resultant data to derive indices of blood-to-brain transfer. There are two advantages to this approach. First, measurements can be made repeatedly in the same animal; for instance, they can be made before drug treatment and then again after treatment to assess efficacy. Secondly, MRI studies can be multiparametric. That is, MRI can be used to assess not only a blood-to-brain transfer or influx rate constant (K_i or K_1) by DCE-MRI but also complementary parameters such as: 1) cerebral blood flow (CBF), done in our hands by arterial spin-tagging (AST) methods; 2) magnetization transfer (MT) parameters, most notably T_{1sat} , which appear to reflect brain water-protein interactions plus BBB and tissue dysfunction; 3) the apparent diffusion coefficient of water (ADC_w) and/or diffusion tensor, which is a function of the size and tortuosity of the extracellular space; and 4) the transverse relaxation time by T_2 -weighted imaging, which demarcates areas of tissue abnormality in many cases. The accuracy and reliability of two of these multiparametric MRI measures, CBF by AST and DCE-MRI determined influx of Gd-DTPA, have been established by nearly congruent quantitative autoradiographic (QAR) studies with appropriate radiotracers. In addition, some of their linkages to local pathology have been shown via corresponding light microscopy and fluorescence imaging. This chapter describes: 1) multiparametric MRI techniques with emphasis on DCE-MRI and AST-MRI; 2) the measurement of the blood-to-brain influx constant and CBF; and 3) the role of each in determining BBB permeability.

Keywords

Apparent diffusion coefficient; Arterial spin tagging; Blood-brain barrier; Cerebral blood flow; Cerebral ischemia; Gd-DTPA; Hemorrhagic transformation; Influx constant; Look-Locker; Magnetic resonance contrast agents; Magnetization transfer; Patlak plot; Quantitative autoradiography; Rat; T_1 ; T_{1sat} ; T_1WI ; T_2 ; TOMROP

1. Introduction

The blood-brain barrier (BBB) is a structural and functional feature of the cerebral microvascular system that tightly regulates the flux of substrates into and out of the brain (1). The “barrier” in this system is mainly formed by the continuous series of tight junctions that join the endothelial cells of the brain microvessels and close the paracellular pathway. The lack of fenestrae and reduced numbers of caveolae within the endothelial cells also contribute to barrier function. Other constituents of the BBB complex are the basal lamina (BL), the pericytes that are embedded in the BL, and a surrounding cuff of astrocytic end-feet (1).

Spontaneous, mechanical or drug-induced thrombolysis after cerebral ischemia can lead to either partial or complete restitution of cerebral blood flow (CBF) resulting in reperfusion of the ischemic regions. However, if not begun within a very short time window after stroke onset, reperfusion can induce opening of the BBB and a different, more damaging lesion than that caused by permanent occlusion (2). This acute BBB damage can worsen over time, leading to hemorrhagic transformation (HT) which can be fatal (3). Furthermore, the risk of HT can increase as much as 10-fold with tPA treatment (4). Presently, apart from stroke onset time, no other reliable indicator(s) of the risk of HT are available. The heightened threat of HT associated with thrombolysis has, thus, restricted the widespread application of this vital therapy.

Some non-invasive techniques are available for characterizing acute stroke in humans. Traditionally, computed tomography (CT) has been the imaging modality of choice. More recently, multiparametric MRI, which combines two or more MRI techniques such as diffusion-weighted imaging (DWI) and perfusion-imaging (PWI), has been used to identify the penumbra region of the ischemic lesion. This region is characterized by a so-called perfusion-diffusion mismatch and is thought to be made up of tissue that is injured, but not irreversibly, and can be saved by prompt reperfusion (5). This approach has been employed to select patients for tPA therapy within and beyond the ‘3-hour-after-stroke-onset’ time window (6,7). Adding to the repertoire of multiparametric MRI, MR contrast agent (MRCA)-enhanced imaging has also been shown to delineate ischemic regions with acute BBB alteration (8,9). As alluded to above, detection of acute BBB opening in stroke is important since these regions have been shown to develop HT later either with or without tPA in both experimental models (3,10) and humans (11).

Despite the capability of MRI to detect areas of BBB opening, precise localization and quantification of such damage in stroke by dynamic contrast agent-enhanced MRI (DCE-MRI) was still lacking until several years ago. Originally, quantification of DCEMRI data was achieved by deconvolution to determine a transfer constant, K^{trans} , that was highly model-dependent (12). We have now developed a novel method for localizing *and* quantifying DCE-MRI data using Patlak plots to obtain the blood-to-brain transfer or influx rate constant (K_i) of Gadolinium- diethylenetriaminepentaacetic acid (Gd-DTPA) and tested the spatial and quantitative accuracy of these estimates with radiotracers and QAR (13). This 25-year-old Patlak plot technique, recently introduced by us to DCEMRI, has now been replicated successfully by other labs using various magnet and console combinations in both experimental models and human disease (see Note 1).

¹A major caveat to the methods presented in this chapter is that conditions described were optimal to the magnet and console used and the particular set of MR sequences that were developed for this system. It should be noted, however, that they are easily adaptable to other MRI systems as well.

Patlak and co-workers developed a treatment of tracer uptake over a period of circulation by taking into account tissue and blood concentrations of the tracer at a number of points (minimally more than four) after injection (14,15). This multiple-time graphic method yields a curve of tissue uptake as a function of blood tracer level and time. The slope of the linear part of this curve represents K_i , which is the most useful and accurate of the several blood-brain transfer constants that can be linked to capillary permeability. These data and the Patlak plot approach for analyzing MRI findings were confirmed with the images and K_i results obtained with ^{14}C -sucrose-QAR some minutes later in the same animals (13). This work was further extended to establish the spatial resolving power of DCE-MRI after a bolus MRCA injection by comparing these results to the QAR images of ^{14}C -aminoisobutyric acid (AIB) distribution gained concurrently in the same rats (16).

Despite employing a very precise MRCA infusion technique, it is possible not to see contrast-enhancement in spite of BBB damage in stroke since adequate blood flow is required for the delivery of the MRCA to the tissue of interest. In acute stroke, CBF is the first function to be affected and low flow may result in little or no delivery of MRCA to the tissue. For this reason, an MRI technique for estimating the leakiness of the BBB that did not involve an MRCA was sought. In one series of experiments, the ability of magnetization transfer (MT)-MRI methods was tested against classical Gd-DTPA enhanced MRI to look for alternate quantitative MRI signatures that indicated BBB opening (17). The MT-based indices tested were: T_1 and $T_{1\text{sat}}$. The regions with increased T_1 and $T_{1\text{sat}}$ changes accurately reflected BBB changes as confirmed by DCE-MRI, QAR and dual-contrast enhanced MRI (18). The MT-MRI could also be used to segment and measure tissue damage (17).

Together these techniques represent a powerful array of minimally invasive and non-invasive quantitative MRI methods for accurate localization and quantification of tissue and BBB damage in stroke (Fig. 1) and brain tumors. The multiparametric MRI approach can also be applied to study other pathologies such as multiple sclerosis, Parkinson's disease and neurotrauma. The greatest advantage is that it can be repeatedly applied in the same subject within several hours on the same day or employed as follow up across different days. These measures are expected to aid in cerebrovascular disease characterization and its response to treatments.

2. Materials

2.1. Middle Cerebral Artery Occlusion (MCAO) Model

1. Young adult Wistar rats, weighing 300 g.
2. Sterile surgical instruments, including microvascular clips (Codman, Raynham, MA), skin clamps.
3. Sterile 4–0 nylon suture, about 2 cm in length with its tip rounded using a heat source.
4. Sterile silk 4–0 sutures with needles.
5. Sterile 1 ml syringes (Becton Dickinson, Rutherford, NJ, USA).
6. Sterile 23 G needles (Becton Dickinson, Franklin Lakes, NJ, USA).
7. PE–50 catheters with beveled, rounded tips, about 100 cm long for venous and arterial lines. The other end of the catheter is connected to a blunted 23 gauge needle attached to a 1 ml syringe (Becton Dickinson, Sparks, MD, USA).
8. Betadine solution and alcohol swabs.

9. A small, animal hair clipper.
10. Sterile saline (500 ml) bags, each containing 50 IU heparin.
11. Anesthesia apparatus and small-animal nose cones for isoflurane anesthesia (Baxter Healthcare Corp., Deerfield, IL) and a lidocaine (Abbott Labs, N. Chicago, IL) for irrigation.
12. A water-recirculated heating pad (Kent Scientific Corp., Torrington, CT, USA).
13. Sterile drapes, masks, gloves, gauge.
14. Operating microscope (Carl Zeiss, Germany).
15. A manual or programmable syringe pump (Model 944, Harvard Apparatus, South Natick, MA).

2.2. 9L Cerebral Tumor Implantation Model

1. Male Fischer-344 rats, weighing between 200-240 g (Charles River Breeding Laboratories, Wilmington, MA).
2. Stereotaxic equipment with two positioning holders for a dental drill and a Hamilton syringe each (David Kopf Instruments, Tujunga, CA).
3. Sterile -surgical instruments including scalpels, silk sutures, bone wax.
4. A sterilized 10 μ L Hamilton syringe with a beveled tip.
5. A foot-operated dental drill (Foredom Series F, Bethel, CT).
6. Injectable anesthetics: Cocktail containing Ketamine (Abbott Laboratories, N. Chicago, IL; 80 mg/kg) and Xylazine (Phoenix Scientific Inc., St. Joseph, MO; 10 mg/kg).
7. A tube of ophthalmic ointment (AKWA Tears, Akorn Inc., Buffalo Grove, IL).
8. Sterile drapes, masks, gloves, gauge.
9. A source of immortalized glioblastoma cells (Dept. of Radiation Oncology, Henry Ford Hospital, Detroit, MI).
10. Eagle's minimum essential medium (Baxter Healthcare, Deerfield, IL), supplemented with 10% fetal calf serum, 1% multivitamins and 1% nonessential aminoacids.
11. Trypsin (GIBCO, Grand Island, NY, USA; 0.05% stock solution diluted 1:1 with PBS).

2.3. Magnetic Resonance Imaging

1. A dedicated small animal magnet (Magnex Scientific, Inc.; Abingdon, UK) (*see* Note 2).
2. Magnet-compatible imaging sequences and consoles (Bruker Biospin MRI, Billerica, MA, USA).

²The radiofrequency (RF) coil and animal holder assembly for MRI were designed so that the RF coil section remained fixed within the bore of the magnet and the animal holder assembly could separately be removed if necessary. This setup allowed the animal to be removed from the magnet for reperfusion and then returned to exactly the same position within the magnet.

3. Offline, post-processing hardware (SUN Microsystems, Inc., Santa Clara, CA) and software (Eigentool, Henry Ford Health System, Detroit, MI; C-program based Unix Shell scripts).
4. A commercially available or lab-made Gadolinium-based MR contrast agent such as Magnevist (Berlex, Montville, NJ, USA; 0.1 mmol/Kg).

3. Methods

Animal studies were performed in accordance with National Institutes of Health guidelines for animal research and were approved by the Institutional Animal Care and Use Committee.

3.1. Experimental Models

3.1.1. Middle Cerebral Artery Occlusion (MCAO) Model—In order to develop and test BBB permeability measurement techniques, it was necessary to utilize an animal model capable of producing a focal region of brain tissue with BBB opening. For this purpose we used a model of transient focal cerebral ischemia in rats via intraluminal occlusion of the MCA with a nylon filament (3,10).

1. Rats are anesthetized and placed supinely on the water-recirculated heating pad and the core temperature is kept at 37 ± 1 °C throughout all surgical and MRI procedures using a feed-back regulated rectal probe.
2. The neck area is cleaned with alcohol swabs and betadine solution and shaved to expose skin.
3. A 2 cm longitudinal incision is made at the midline of the ventral aspect of the neck and the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) are exposed under the operating microscope. Care should be taken to avoid injuring the vagus nerve.
4. The ICA is further dissected to identify the pterygopalatine branch and intracranial ICA and a 5–0 silk suture is loosely tied at the origin of the ECA and the distal end of the ECA is ligated.
5. The CCA and ICA are temporarily clamped with microvasculature clips.
6. The 2 cm long, 4–0 surgical nylon filament, with a rounded tip round is introduced into the ECA lumen through a small puncture and the suture at the origin of the ECA tightened around the intraluminal nylon filament to prevent bleeding and the microvascular clips are removed.
7. The nylon filament is gently advanced from the ECA into the lumen of the ICA, a distance of 18.5 – 19.5 mm according to animal weight, until the tip of the filament blocks the origin of the middle cerebral artery (MCA) and the microvascular clip is released.
8. The skin incision is temporarily closed with either loose silk sutures or skin clamps. After MR quantification of occlusion effects, the rat is taken out of the magnet and reperfusion is performed at the desired time as indicted in **step 9**.
9. The skin sutures/clamps are opened and the occluding filament is withdrawn until its tip is visible in the lumen of the ECA and is no longer occluding the MCA or restricting flow in the ICA and the neck incision is closed.

3.1.2. 9L Gliosarcoma Brain Tumor Model—In brain tumors, BBB opening can be due to both BBB damage and increased angiogenesis which is typically seen at the periphery of solid experimental tumors. Owing to the tremendous leakiness of the vessels, it is also necessary to correct the Patlak model for MRCA backflux. The optimal experimental conditions that accurately reflect native leakage and indices of response to putative treatments were developed (19,20) using the 9L gliosarcoma model (21).

1. The tumor cells are maintained as exponential cultures in Eagle's minimum essential medium (see **Subheading 2.2, #11**) (21).
2. Immediately prior to each implantation, cells are trypsinized, resuspended and a final dilution of 2×10^6 cells per mL is made in Eagle's medium without serum.
3. The rat is anesthetized with an intraperitoneal injection of a cocktail of ketamine and xylazine and positioned in the stereotaxic head frame.
4. The scalp is cleaned with alcohol swabs and betadine and hair is shaved from the frontal surface of the scalp with electric clippers and ophthalmic ointment is applied to the rat's eyes to keep the eyes moist.
5. An incision is made along the midline on the dorsal surface of the head to expose the frontal and temporal bones.
6. The position of the injection site is marked using one positioning arm of the dental drill: 2.5 mm anterior to the bregma and 2.0 mm to the right of the midline.
7. A 1.0-mm burr-hole for implanting tumor cells is drilled at this point through the skull without breaking the dura.
8. The Hamilton syringe fitted with a 26-gauge needle is filled with 5 μ L of the cell suspension and fixed to the other stereotaxic positioning arm. Its tip is positioned over the burr-hole. The arm is then lowered to a depth of 3 mm and raised by 0.5 mm. The 5 μ L volume is slowly injected to implant the tumor cells.
9. The syringe is left in place for 5 min and then gently retracted. The burr-hole is sealed with sterile bone wax and the skin sutured with sterile silk sutures.
10. Tumors are allowed to grow for up to 14-15 days post-implantation before MRI.

3.1.3. Femoral Arterial and Venous Cannulation—

1. Anesthetize the rat, place it in a supine position on the heated rubber mat. Extend its legs and fix them to the operating base with adhesive tape.
2. Clean the skin on either left or right groin with alcohol swabs and shave hair in this region and apply betadine to the skin surface.
3. Make approximately 1 cm long, oblique (along the direction of extended leg) skin incision in the groin.
4. Expose the femoral artery and vein, separate them and clean the fascia.
5. For each vessel, a small length (~10 cm) 4-0 silk suture is put loosely around the proximal part and the ends of the suture are weighed down by a hemostat to pull the vessel taut. The distal end (approximately 1 cm apart) is ligated using a 4-0 silk suture with enough length for two more knots.
6. An opening (approximately one-half the vessel diameter) is made in the middle of this 1 cm segment of the vessel.

7. The beveled end of a PE-50 catheter is introduced into this opening, threaded about 1.5 cm proximally without kinking the vessel and the proximal suture is used to ligate the vessel over the catheter. Care must be exercised not to ligate beyond the catheter tip to avoid occluding the vessel.
8. The remaining part of the distal suture is used to tie the catheter to the vessel to prevent it from slipping out.
9. The catheters are tested for smooth blood flow. Blood samples can be now collected or the vessels can be used for physiological monitoring, drug injections etc.

3.2. Basic MRI Experimental Procedures

1. The rat is placed in a plastic holder following the surgical procedures. It can be either in a supine or prone position depending on the design of the MR coil. This holder should have provisions for supporting the animal's body and for administering anesthetic gases if they are being used.
2. A feedback regulated water blanket and non-magnetic stereotaxic ear bars minimize head movement during imaging.
3. The animal holder is placed inside a 5 cm diameter quadrature driven transmit/receive birdcage coil tuned to the resonant proton frequency of ~300 MHz and the entire assembly is placed inside the MRI unit (*see Note 2*).
4. Scout images are obtained to adjust the position of the animal's head until the central image slice is located at bregma.
5. Receiver and Transmitter coils are tuned to the frequency of the sample using the 'wobble' mode of the setup.
6. Shimming, and setting up central frequency, RF gain and receiver gain are done either automatically or manually.

3.2.1. Measurement of Cerebral Blood Flow (CBF)—Blood flow, an important function of the cerebral vascular system, can be measured at the local level by MRI. It is important that CBF be measured in studies of BBB permeability because the actual parameter determined in the latter experiments is an influx rate constant, K_i , depending not only on capillary permeability (P) and surface area (S) but also CBF. To get from K_i to PS product, the physiological expression of capillary permeability, the rate of blood flow is required. Hence, CBF is estimated in all of our MRI experiments by PWI using the arterial spin tagging (AST) technique as follows.

1. Cerebral perfusion can be estimated using AST with a variable tip-angle gradient-refocused echo (VTA-GRE) imaging technique (24).
2. In this procedure, the protons within the blood passing through the arteries of the neck are magnetically inverted thereby serving as an autologous label. The decrease in the net magnetization of the perfused tissue, a function of the rate of blood flow, is then regionally detected in the brain.
3. AST uses gradient and frequency offsets to create a plane of inverted spins in the neck of the animal approximately 18 mm below the center of the imaging plane. AST is performed by labeling inflowing arterial protons via a continuous wave (CW) RF adiabatic inversion pulse applied in the presence of a magnetic field gradient.

4. Labeling of inflowing arterial water protons is achieved via an axial gradient of ± 0.3 kHz/mm using a CWRF pulse at a power of 0.3 kHz at a frequency offset of ± 6 kHz followed by a VTA-GRE sequence with repetition time (TR)/echo time (TE)=11ms/5ms (24).
5. To allow for equilibration of inverted protons in the imaging plane, the inversion pulse is turned on for a period of 4.5 s preceding the production of each VTA-GRE image.
6. A corresponding set of control scans is acquired using gradient and frequency offsets that put the inversion plane 18 mm above the imaging plane (i.e. outside the head).
7. The images are acquired in sets of 4 with the frequency offset and gradient polarities permuted through all 4 combinations to remove any gradient asymmetries in the axial direction and to balance off-resonance MT effects. The images are acquired over a 32 mm field of view (FOV) and reconstructed using a 64×64 image matrix.

3.2.2. Magnetization Transfer (MT) Parameters—This involves the adiabatic transfer of magnetization between the free or mobile pool of protons, which in biological specimens is mostly water, and the bound or immobile pool of protons, mainly those associated with hydration layers of water surrounding large macromolecules and proteins. Of importance, changes in the MT parameters often correlate well with BBB opening, probably as the result of increased tissue water (i.e. vasogenic edema). The MT related parameters are M_0 , M_{0sat} , T_1 , T_{1sat} , K_{for} and the magnetization transfer ratio (MTR). Calculation of the apparent forward magnetization transfer rate, K_{for} , requires estimates of T_1 in both the absence and presence of off-resonance irradiation of the “bound” proton fraction (T_1 and T_{1sat} , respectively).

1. Estimates of T_1 and T_{1sat} are obtained using the Phase Incremented Progressive Saturation (PIPS) method (TR/TE= 40/7 ms, tip angle $\sim 18^\circ$) (25).
2. The sequence is run in sets of two to produce estimates of M_{0sat} , T_{1sat} , M_0 , and T_1 . To estimate M_{0sat} and T_{1sat} , a CW RF saturation pulse of approximately 0.3 KHz amplitude, off-resonance by 6 KHz, is turned on for 4.5 s before the beginning of each image of the PIPS sequence, and for 30 ms (of the 40 ms TR) between each gradient-echo line in k-space to partially saturate the macromolecular proton pool.
3. The value of K_{for} is calculated from the equation:

$$K_{for} = \frac{1}{T_{1sat}} \left(1 - \frac{M_{0sat}}{M_0} \right) \quad (1)$$

where K_{for} represents the product of $1/T_{1sat}$ and the magnetization transfer ratio (MTR = $1 - M_{0sat}/M_0$), which is often used alone to examine MT effects (If the transfer of magnetization decreases, then T_{1sat} may have increased or MTR decreased; K_{for} from Equation 1, thus, provides a useful summary of MT effects). The total imaging time for both variations of the PIPS sequence is approximately 24 min.

3.2.3. Measurement of T_2 —The biophysical meaning of T_2 , the transverse relaxation time, is unclear, but changes in T_2 within areas of the brain often indicate underlying tissue injury or dysfunction with elevated T_2 values being highly correlated with increased tissue water content.

1. Sets of T_2 -weighted image data are obtained using a multi-slice Carr-Purcell-Meiboom-Gill (CPMG) sequence (TR=1200 ms, TE=30, 60, 90 and 120 ms, with interleaved slice acquisition, 13 slices, slice thickness=1 mm). Total imaging time for the T_2 WI series is approximately 13 min.
2. T_2 maps (quantitative images obtained by pixel-by-pixel processing of raw imaging data) are produced from a straight line least squares estimate of the slope from a plot of the natural logarithm of the normalized image intensity $[\ln(S/S_0)]$ versus TE.

3.2.4. Measurement of the Apparent Diffusion Coefficient of Water (ADC_w)—

The apparent diffusion coefficient of water in brain is a function of the size and tortuosity of the extracellular space, the rate of water exchange between intracellular and extracellular fluid (ECF), the volume of intracellular water, and the rate of bulk flow of extracellular and edema fluid, if present.

1. A pulsed gradient spin-echo imaging sequence with progressively incremented diffusion weighting (b -value) is used to measure ADC_w (TR/TE = 1500/40 ms, b -value = 0, 400, 800 s/mm^2 , 128×64 image matrix and interleaved slice acquisition). The total scan time for this diffusion-weighted imaging (DWI) is approximately 10 min for the complete data set.
2. Maps of ADC_w are produced from a straight line least squares estimate of the slope from a plot of $[\ln(S/S_0)]$ versus gradient b -value.

3.2.5. Look-Locker MRI Quantification of T_1 after Gd-DTPA Injection—

Gadolinium-DTPA changes the longitudinal relaxation rate (symbolized by R_1), of the nearby water protons. The MRCA concentration is proportional to R_1 (R_1 is the inverse of the longitudinal relaxation time, T_1) when this parameter is quantitatively determined.

1. Rapid quantitative estimates of brain tissue T_1 relaxation times are necessary to accomplish an estimate of MRCA tissue concentration as it varies with time, and can be obtained using an imaging adaptation of the Look-Locker method (26).
2. The technique employs the T_1 by Multiple Read-Out Pulses (TOMROP) sequence (27) to produce efficient and unbiased pixel-by-pixel estimates of T_1 (28).
3. Baseline high-resolution T_1 -weighted spin-echo images are collected prior to Gd-DTPA injection. Then, after obtaining one or two baseline L-L T_1 estimates, Gd-DTPA is injected as a bolus into the femoral vein in <5 s and L-L data sets are acquired sequentially at approximately 2.5 min intervals for up to 30 min (13).
4. Data are obtained for five interleaved 2 mm thick, slices. At the conclusion of the L-L series, a final T_1 -weighted multislice SE image set is obtained.

3.3. Estimating Transfer Constants by Patlak plots

1. The L-L T_1 maps over a series of time points are acquired as described above to estimate arterial plasma and tissue concentrations of the MRCA.
2. The plasma and tissue MRCA concentrations are determined by measuring MRCA induced T_1 changes in the superior sagittal sinus (SSS) and brain-tissue,

respectively according to the relationships: $C_{pa}(t_n) \propto \frac{\Delta R_{1a}(t_n)}{(1 - Hct)}$ and $C_{tis}(t_n)$

$R_{1tis}(t_n)$, where $R_{1tis}(t_n)$ and $R_{1a}(t_n)$ are differences in $R_1 (=1/T_1)$ measured in the tissue and sagittal sinus, respectively, at time point $t = t_n$ and $t = 0$ (baseline or pre-CA time point) and Hct is the hematocrit (see Note 3)

3. Patlak plot technique is employed to estimate K_i and V_p+V_o by graphing

$$\frac{(1 - Hct) \Delta R_{ltis}(t)}{\Delta R_{la}(t)} \text{ versus } \frac{\int_0^t \Delta R_{la}(t) dt}{\Delta R_{la}(t)} \text{ as described below.}$$

The solution of the equations for the Patlak model of blood-brain exchange is given

$$\text{by the expression (15): } \frac{C_t}{C_{pa}} K_i \frac{\int_0^t C_{pa}(t') dt \text{ prime}}{C_{pa}(t)} + (V_p + V_o).$$

4. A graph of the ratio of the tissue CA concentration at the times of sampling (C_t) to the arterial plasma concentration at the respective times (C_{pa}) versus a concentration weighted time parameter referred to as “plot time” (integral of C_{pa} over time up to the time point of measurement divided by C_{pa} at that time) can be drawn to estimate K_i and V_p+V_o (Fig. 2). In MRI, linear least squares estimates of K_i and V_p+V_o are performed pixel-by-pixel to construct the corresponding maps (see Note 4).

3.4. MRI Data Analysis and the Selection of Regions of Interest

1. The MRI data are processed offline using a system such as a SUN workstation.
2. A semi-automated segmentation algorithm is employed to minimize user bias in identifying tissue areas of MR abnormality indicative of processes such as BBB opening or hyperemia.
3. The MRI data are then reconstructed and baseline corrected using in-house software before applying the segmentation procedure. Most of the data from our studies were obtained from what is referred to as the central slice, 2.0 mm thick and extending from bregma +0.15 mm to -1.85 mm. Comparable image analyses techniques can be applied to adjacent slices if necessary and the data merged. One of the several available image segmentation protocols can be used to identify the regions of interest (ROI) (see Note 5).

3.5. MRCA Administration by Step-down Infusion (SDI)

To compensate for the exponential decay of blood MRCA level after a bolus injection, an experimental step-down infusion (SDI) technique that maintains the blood tracer level constant for the duration of DCE-MRI can be used (22). The SDI protocol improves the signal-to-noise ratio, enhances the spatial resolution of the images, and yields a more accurate blood concentration time course (the arterial input function or AIF) of the MRCA (23). For the usual bolus injection method, 60 μ L of the stock solution is diluted to 0.1 ml with normal saline and injected via the femoral vein in 4-5 s. The SDI, however, is done with a syringe pump and a prescribed infusion schedule (Table 1).

³The term (1-Hct) is used to adjust the Gd-DTPA concentration measured in whole blood to the concentration in plasma, since Gd-DTPA distributes only in plasma. In each experiment, the arterial hematocrit (Hct) should be measured prior to injecting Gd-DTPA, and the resulting value used for the graphical estimation. It has been assumed that the constant of proportionality (i.e., the relaxivity) between concentration and R_1 does not change across tissues, although there are exceptions to this assumption (29).

⁴Experimental data demonstrating the applicability of the model and the Patlak plot were produced by Blasberg et al. (30) with radiotracers such as ¹⁴C-AIB and ⁵¹Cr-DTPA. It should be noted that the abscissa of the Patlak plot has the units of time but is an arterial concentration weighted time.

⁵For our earlier work, we identified and segmented the ischemic brain regions with BBB injury based on direct subtraction of pre- and post-MRCA spin-echo T_1 -weighted image (T_1 WI) data or application of the Iterative Self-Organizing Data Analysis Technique Algorithm (ISODATA) to the temporal Gd-DTPA L-L T_1 WI data set (Fig. 1) (31). Another method we have recently employed for segmenting ischemic brain regions with BBB opening is based on Patlak plot-derived F-test maps (Fig. 3) (19).

1. For the infusate, 240 μL of the stock solution is diluted in 4.0 mL of saline (the osmolality of the Gd-DTPA preparation is very high (>1000 mOsm/kg), and this dilution brings it into the physiological range for infusion).
2. Approximately 3.5-3.6 mL of infusate is used per step-down experiment following the protocol given in Table 1. The rest of L-L T_1 WI methods are identical to those explained above.

3.6. Estimating acute treatment efficacy in a brain tumor model

1. At the desired time-point after tumor implantation, the rat is anesthetized and a venous arterial and cannulations are performed as described above.
2. It is then positioned in the MRI holder and placed inside the magnet.
3. The scout images and T_2 images are obtained to locate and measure the tumor.
4. The chosen contrast media (normally, a large MRCA) is then injected and SE- and L-L T_1 WI-images are obtained as described.
5. The drug being tested is injected at the appropriate dose after insuring the return to base-line of MRCA signal.
6. After waiting for drug action, one more MRCA injection is given and another set of SE- and L-L T_1 WI- images are obtained.
7. The contrast enhancement before and after drug injections are compared quantitatively to assess drug effects (Fig. 3; see Note 6).

3.7. Sizing BBB Opening with Two Different MRCAs

1. The MCAO model with acute reperfusion is employed for this purpose. Therefore, perform the MCA occlusion and reperfusion following the protocols given in **Subheading 3.1.1**.
2. Measure, following the MRI protocols, the ischemic status of the tissue and the extent of reperfusion.

⁶The well-characterized, solid, 9L brain tumor model lends itself nicely to the identification of drug effects on the leaky BBB and the speed of onset of such effects. Of the several parameters examined, K_i turned out to be the most sensitive to the effects of dexamethasone (Fig. 3) (20). Acute effects of dexamethasone on brain tumor permeability had been shown by earlier studies (32) and this provided us with a known response and an acceptable drug-tumor system for examining the sensitivity of our test-retest protocol.

- a. For a drug efficacy study, the MRCA and the protocol should have several physiological and pharmacological features and constraints. First, it must be cleared from the blood fairly rapidly after bolus administration so that the second test is not strongly influenced by CA remaining from the first test. Secondly, it should have a characteristic transfer rate to the tissue that is much slower (ideally a factor of 10 or more) than the tissue sampling rate. This latter constraint is necessary because the rate of transfer has to be inferred from the concentration-time trace, and this is impossible if the CA equilibrates in the tissue on a time scale that is rapid compared to the sampling rate.
- b. A small MRCA such as Gd-DTPA (~560 Da) diffuses rather quickly through the blood-tumor barrier of the 9L model, which is fairly leaky at this stage. This makes it a less than ideal contrast agent for this tumor model with respect to the second constraint above. In addition, the linear phase of uptake can be very short or even lacking on a simple Patlak plot of Gd-DTPA data. Therefore, larger MRCAs that permeate the brain-tumor barrier more slowly than Gd-DTPA, do not accumulate greatly, but move appreciably in the field of observation are more useful.
- c. In a typical experiment, the influx constant for an MRCA is determined first and then the drug is injected. Depending on the expected time-course of drug action, the MRCA is injected again at an appropriate time and L-L T_1 measurements are made thereafter as done in the test period. The pre-drug results are then compared to post-drug results.
- d. Though simple in design, the constraints listed above need to be considered in choosing the contrast agent, but it is quite easy to make successive studies on the same animal when appropriate care has been taken in selecting the proper combination of MRCA and drug response time. The time of retesting after drug administration, can be varied among groups of animals and the period of efficacy determined over one to many hours after treatment.

3. Quantify the spatial extent of the BBB opening by quantitative Patlak- K_i maps in the ischemic hemisphere using Gd-DTPA-enhanced L-L T_1 WI. Wait for the Gd-DTPA signal to return to baseline.
4. Then quantify BBB opening and generate an identical data set using a larger MRCA such as Gd-BSA following the same protocols.
5. Compare the spatial enhancement patterns and K_i maps for the two contrast agents to determine the differences, and, thus, relative sizes of BBB opening in the ischemic tissue (Fig. 4; see Note 7).

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⁷Blood-brain barrier opening is generally not an all-or-none phenomenon but is graded with a small tracer leaking at a particular time after ictus but not a larger one. A protocol similar to the test-retest one just presented can be used to measure blood-to-brain influx of two MRCAs of different sizes within a short duration of each other and obtain nearly congruent results to evaluate the “size” of the opening.

- a. The same constraints and consideration indicated above in the test-retest protocol apply to a BBB sizing study. Perhaps the most limiting one in this instance is the plasma half-life of the MRCA. This means that the first MRCA injected is the one with the shorter half-life and the longer half-life one is given sometime later (18).

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Figure 1.

A representative data set showing multiparametric MRI-derived maps obtained before (I1t) and after reperfusion (R1t) from an animal subjected to 3 h of transient focal ischemia. Top (left to right): cerebral blood flow (CBF), apparent diffusion coefficient (ADC_w) and transverse relaxation time (T_2) maps. Middle (left to right): spin–lattice relaxation times in the absence and presence of off-resonance saturation (T_1 and T_{1sat} , respectively), the forward rate of magnetization transfer (K_{for}), and magnetization transfer ratio (MTR) maps. Bottom row: A corresponding ISODATA segmentation theme map to demonstrate the ROIs that were selected to represent ischemic tissue with (red and green) and without (yellow) BBB disruption (left). To the right, are a corresponding ^{14}C -AIB autoradiographic image and a cresyl violet stained histologic section that were used to confirm acute BBB damage and the region of ischemic damage, respectively. (From Knight et al. *Magn Reson Med.* 2005;**54**:822-832)

Figure 2.

Representative Patlak plots of the ratio of brain tissue (C_{tis}) concentrations of Gd-DTPA to plasma [$C_{pa}=C_a(t)/(1-Hct)$] vs. concentration ratio-stretched time ($t_{stretch}$) for three brain regions of interest (ROI) from one rat. The Gd-DTPA K_i values were obtained from the slope of the lines. Among the regions with leaky capillaries, the influx appeared to be greater in the preoptic area (PoA) than the striatum. The slopes in these two regions were significantly different from zero and indicate an appreciable, but small, Gd-DTPA influx. The slope of the line was flat (and statistically not different than zero) for the contralateral ROI and indicates a normal K_i . The units on the abscissa are plasma concentration ratio-stretched time, not real time (which was about 24 min in this case). (From Knight et al. *Magn Reson Med.* 2005;**54**:813-821)

Figure 3.

Vascular parameters pre- (left, test) and post-dexamethasone (right, re-test) administration. A: Vascular volume v_D , B: Transfer constant K_1 [min^{-1}], C: Efflux constant k_b [min^{-1}], D: F-test for Model 3 vs Model 2, with a high value resulting in rejection of Model 2. Only those regions in C with high F-test values have valid results for the estimate of k_b . A widespread decrease in K_1 and k_b are easily visualized. Less visible is a moderate decrease in v_D . Bright spots in the maps of v_D correspond to vascular pools. Note the decrease in the F-test from pre- to post-dexamethasone studies. (From Ewing et al. *J Magn Reson Imaging* 2008;**27**:1430-1438)

Figure 4.

A representative collage of Gd-DTPA and Gd-BSA-Evans Blue (EB) enhancements during MRI, corresponding T_{1sat} maps and fluorescence images from one experiment. A – Gd-DTPA enhancement in a rat 24 hours after 3 hours of MCA occlusion. A large area of brightness is seen in the preoptic area and striatum. The small, bilateral, bright regions below this enhancement are parts of medium eminence and hence, naturally leaky. Contrast enhancement in such regions, ventricles (large arrows), and pial vasculature surrounding the brain were routinely observed. B – Subsequent Gd-BSA-EB enhancement in this rat. It is a much smaller area. Other normally leaky regions are also visible. In C, the T_{1sat} map for this slice is shown in gray scale. On the calibration bar on the left, hyperintense/bright areas indicate increased values. The ventricles and circumventricular organs appear bright on this map due to the inherent sensitivity of T_{1sat} to water/proton shifts. The low-magnification, reconstructed fluorescence image shown (D) has extravascular red fluorescence in the same regions of interest as the Gd-BSA-EB-enhancing area in B. The greenish-yellow hue in the rest of the vasculature is due to the combination of red (EB) and green (fluorescein isothiocyanate–dextran) fluorescence. This image was constructed by collecting the coronal brain section as a series of low magnification (X2.5) images and tiling them together. (From Nagaraja et al. *Stroke*. 2008;**39**:427-432).

Table 1

The step-down infusion protocol developed and employed in the authors' laboratories. (From Nagaraja et al. Magn Reson Imaging. 2007;**25**:311-318)

Rate (ml/min)	Pump speed (%)	Time	Duration (min)	Volume (ml)
0.68	100	0-30 s	0.5	0.34
0.53	77.7	30-60 s	0.5	0.26
0.39	57.4	1-2 min	1.0	0.39
0.28	41.6	2-3 min	1.0	0.28
0.22	33.3	3-4 min	1.0	0.22
0.19	28.7	4-5 min	1.0	0.19
0.17	25.0	5-7 min	2.0	0.34
0.14	20.4	7-10 min	3.0	0.42
0.12	17.6	10-15 min	5.0	0.60
0.10	14.8	15-20 min	5.0	0.50
Total volume infused =			3.54 ml	