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A Continuous-Infusion Dynamic MRI Model at 3.0 Tesla for the Serial Quantitative Evaluation of Microvascular Proliferation in an Animal Model of Glioblastoma Multiforme

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Abstract

Purpose—To develop a continuous-infusion dynamic MRI technique to characterize tumor-associated microvascular proliferation (MVP) in a rat brain model of glioblastoma multiforme.

Theory and Methods—The proposed model assumes effects due to tumor-associated MVP (e.g., vascular permeability, K^{trans} ; intravascular plasma fraction, v_p) cannot be individually separated and solves for a single parameter (k^{vasc}) that quantifies the T_1 -weighted contrast-enhancement from dynamic images acquired during continuous contrast agent (CA) infusion. Untreated C6 tumor-bearing animals ($N=6$) were serially imaged on post-op days (PODs) 14 and 18 with a 3T clinical scanner utilizing a dynamic spatial and temporal resolution of $0.38 \times 0.38 \times 1.5$ mm³ and 3.47 s, respectively.

Results—An association was present between PODs 14 and 18 for median tumor k^{vasc} (Pearson's $r=0.94$, $P=0.0052$) and CA concentration ([CA], derived from pre- and post-contrast R_1 maps; $r=0.94$, $P=0.0054$). On POD 18, there was a voxel-based association between k^{vasc} and [CA] within each tumor ($0.45 < r < 0.82$, $P < 0.001$). However, voxel-based sub-regions demonstrated a reduced association between k^{vasc} and [CA] ($N=5$; $-0.08 < r < 0.22$, $P > 0.05$) or an inverse association ($N=1$; $r = -0.28$, $P=0.001$) indicating differences between locations of vascular permeability and subsequent CA pooling in tumors.

Conclusions—The continuous-infusion method may provide a quantitative measure for characterizing and monitoring tumor-associated MVP.

Keywords

dynamic MRI; contrast-enhancement; glioblastoma multiforme; animal model

Introduction

Glioblastoma multiforme (GBM) is the most common and most malignant primary brain tumor in adults (1). A key histologic feature of GBM that is absent in normal brain tissues is the occurrence of microvascular proliferation (MVP). Compared to normal cerebral vessels, GBM vasculature has been associated with increased microvessel density (2), high variability in vessel diameter (3), and greater tortuosity (4). The accompanying tight junction formation is also incomplete and results in a compromised blood-brain barrier (i.e., increased permeability) leading to edema and the extravasation of contrast agents into the extravascular extracellular space (EES) during imaging studies of GBM (5–7). MVP as measured by contrast enhancement during radiologic evaluations of GBM has a central role in the diagnosis of new, residual, and recurrent tumors (8–10). Extent of contrast enhancement has been associated with worse clinical outcomes (11–13), while therapies directed at normalization of the vascular structure and function tend to improve progression free survival (14). As new therapies emerge, quantitative imaging methods for the longitudinal evaluation of microvascular-associated attributes may provide mechanistic evidence of efficacy in animal models of GBM prior to usage in clinical trials.

Different pharmacokinetic models that describe the dynamic T_1 -weighted contrast enhancement in the brain have been proposed (15–17). Algorithms based on the bolus delivery of contrast agents (CAs) have been largely utilized during the imaging of human brain neoplasms to measure the endothelial transfer rate constant (K^{trans}), EES volume fraction (v_e), intravascular plasma fraction (v_p), and the CA reflux rate constant (k_e) (18–20). Yet, the abnormal structure and function inherent to tumor-associated MVP poses the risk of under- or overestimation of parameters during attempts to parse contributions between permeability and microvascular structure. Studies have attempted to overcome this limitation by restricting models to first-pass effects during bolus administration of contrast, which has been shown to yield a lower estimate of permeability compared to approaches that utilize the full complement of acquired data points (21). However, temporal resolution of dynamic images (~ 5 s) coupled with a bolus infusion of the CA (~ 4 s) requires either an iterative fitting of non-linear multi-parametric curves with relatively few points that may be susceptible to trapping in a local minimum (22) or the inclusion of additional data points that extend beyond first-pass effects to provide model stability, but also reintroduces interactions between permeability and microvascular structure that the first-pass effect model originally sought to avoid (23). Regardless of approach, multi-parameter models have been shown to yield results across all parameters that vary widely by analytical method (24) supporting previous observations regarding the complexity and uncertainty of separating tumor-associated contrast enhancement into component parts (25).

Beyond the potential limitations associated with pharmacokinetic models, application of dynamic T_1 -weighted contrast enhancement techniques in animal models of GBM presents several technical challenges, particularly on clinical scanners. First, temporal and spatial resolution balanced with signal-to-noise ratio (SNR) has largely driven dynamic acquisitions of the rat brain towards small-bore, high field-strength (i.e., 4.7–12T) animal-specific systems (26–30). The few applications that have occurred on clinical-strength magnets (i.e., 3T) required compromise of either spatial (31) or temporal (32) resolution. Second, use of

custom-made animal coils may improve SNR on clinical magnets (33,34), but implementation of solenoid coils for consistent SNR throughout the brain requires orthogonal positioning of the animal relative to the main magnetic field. As a consequence, bolus injection of paramagnetic CAs induces susceptibility artifacts in vessels desirable for measurement of the arterial input function (AIF; i.e., carotid arteries, superior sagittal sinus (SSS)) and disruption of signal from adjacent tissues (35). Finally, the identification of a suitable AIF during small animal imaging must consider the relatively large in-plane and through-plane acquisition resolutions that are vulnerable to partial-volume effects attributable to size and vessel tortuosity, respectively. As alternatives to measuring plasma CA concentration ([CA]) in intracranial vessels, previous studies have employed standard models of the AIF (36), multi-coil arrangements for concomitant monitoring of contrast arrival in the heart (26), and reference regions models (RRMs) (29). Although the implementation of the latter method has previously introduced an additional variable (i.e., reference tissue permeability) to resolve during curve fitting, the RRM approach permits variability associated with CA infusion between animals while removing the dependence on vessel identification.

In this study, a continuous infusion dynamic T_1 -weighted contrast enhancement model adapted from the Brix method (17) is proposed. The model assumes that intravoxel enhancement represents tumor-associated MVP with abnormal structure and function that cannot be effectively separated. As such, the consequent voxel-based parameter determination derived from tumor-associated dynamic contrast enhancement is denoted K^{vasc} to distinguish from multi-parametric models that utilize the more conventional K^{trans} notation (37). The model also introduces a muscle-based RRM to estimate the AIF required during parameter fitting for a continuous infusion of contrast. Finally, the technique is employed to monitor the effects of MVP in a rat brain tumor model of GBM on a 3T clinical scanner utilizing a comparable spatial and temporal resolution during dynamic imaging as small-bore, high field-strength, animal magnets.

Theory

The proposed two-compartment model is an adaptation of the Brix method (17) that uses a continuous infusion of a CA. The differential equations to describe the mass balance of the model (Fig. 1) in tissues and vessels with normal structure and function are:

$$\frac{dC_p}{dt} = K_{\text{in}} - C_p K_e - C_p K^{\text{trans}} \quad [1]$$

$$\frac{dC_t}{dt} = C_p K^{\text{trans}} \quad [2]$$

where K^{trans} (min^{-1}) is endothelial permeability; C_p (mM) and C_t (mM) are the [CA] in the vascular space and tumor tissue, respectively; K_{in} (mM/min) is the amount of CA being infused over a fixed interval at a constant rate; and K_e (min^{-1}) is the rate of CA elimination

from the plasma volume either through excretion or distribution in tissues. Assuming the reduction of CA from plasma due to lesion loss is insignificant relative to loss from excretion or distribution of CA in other tissues (i.e., $C_p K^{\text{trans}} \ll C_p K_e$), an assumption present in both bolus-based methods (16) and the original Brix method (17), Eq. 1 simplifies to:

$$\frac{dC_p}{dt} = K_{\text{in}} - C_p K_e \quad [3]$$

Solving these differential equations, [2] and [3], with the initial conditions $C_p(0) = 0$ and $C_t(0) = 0$ yields:

$$C_t(t) = K^{\text{trans}} \int_0^t C_p dt \quad [4]$$

$$C_p(t) = \frac{K_{\text{in}}}{K_e} (1 - e^{-K_e t}), \quad [5]$$

respectively. Assuming fast exchange conditions and a linear relationship between the reciprocal of the longitudinal relaxation rate of tissue and CA (16,38,39), the equation for a spoiled GRE (SPGR) sequence is:

$$S(C_t) = GPD e^{-TE(R_2^* + R_2^C C_t)} \sin \theta \frac{1 - e^{-TR(R_1^* + R_1^C C_t)}}{1 - \cos \theta e^{-TR(R_1^* + R_1^C C_t)}} \quad [6]$$

where G is the gain, PD is proton density, R_1^* and R_2^* are relaxivity of tissues prior to CA infusion, and R_1^C and R_2^C are the relaxivities of the CA at 3.0 T and 37°C (40). Using signal enhancement:

$$E(t) = \frac{S(C_t) - S(0)}{S(0)} \quad [7]$$

with Eq. 6 allows determination of [CA] in tissue and plasma for determination of K^{trans} from Eqs. 4 and 5 during a continuous infusion of CA without dependence on G, PD , and R_2^* . In addition, utilizing dynamic data points acquired only during a relatively fast continuous infusion of CA (e.g., <30 s) for parameter determination minimizes non-linear effects associated with fast exchange limit disruption due to interactions between intravascular, interstitial, and intracellular water components (41). Although K^{trans} in healthy tissues represents permeability, intra-voxel contrast enhancement in tumor-associated MVP may be attributable to loss of vascular function, atypical structure, or both. As such, K^{trans} will subsequently be designated as K^{vasc} in the context of abnormal tissues to reflect the model-

associated derivation while also recognizing the ambiguity and complexity in signal origin inherent to MVP.

The identification in the rat brain of a suitable AIF to measure C_p from Eqs. 6 and 7 is challenging as described above. From a spatial-resolution perspective, the SSS represents a viable target; however, factors that affect intracranial pressure (e.g., positioning, tumor mass effect, and edema) may alter blood flow causing variable baseline signal intensity not only between animals, but also from different slices of the SSS within the same animal.

Monitoring contrast enhancement in muscle offers an alternate means to estimate [CA] in plasma, particularly as normal muscle K_m^{trans} (K_m^{trans}) is relatively stable within the context of studying intracranial tumors. Dynamic MRI studies utilizing different techniques have measured K_m^{trans} between 0.02–0.14 min^{-1} (42,43) and previous models of normal mouse muscle have utilized a fixed value of $K_m^{\text{trans}}=0.1 \text{ min}^{-1}$ (44). In the study described herein, setting $K_m^{\text{trans}}=0.1 \text{ min}^{-1}$ allowed the subsequent determination of K_{in} and k_e from Eqs. 4 and 5 by minimizing the sum-of-squares, Q , between muscle and vascular imaging data, S_m and S_v , respectively, and the calculated fit values, T_m and T_v , respectively:

$$Q = \sum_{i=1}^N (S_{m,i} - T_{m,i})^2 + w_v (S_{v,i} - T_{v,i})^2, \quad [8]$$

where

$$w_v = \frac{\overline{S_m}}{S_v}.$$

The coupling of muscle and vascular data is necessary to: 1) prevent unconstrained fitting of muscle data, which is associated with K_{in} and k_e values that yield C_p curves inconsistent with observed imaging changes; and 2) determine the number of data points, N , to include in Eq. 8 since C_t in muscle will continue to rise even after C_p begins to decay. The scaling factor w_v biases the minimization of Q towards the fit of muscle data, while preserving physiologic values of K_{in} and k_e . Of note, K_{in} can be estimated directly based on rate of infusion and calculated plasma volume, however, the fitting of K_{in} may provide a better estimate due to variations in mixing associated with animal positioning, location of IV catheter insertion site in the tail vein, manual injection, and cardiac output.

Methods

Phantoms

Normal Saline (NS) Phantom—gadopentetate dimeglumine (Gd-DPTA; Bayer HealthCare; 0.5 M) was prepared in NS at concentrations of 2, 1, 0.5, 0.25, 0.125, and 0 mM. Each dilution maximally filled a single well in a 24-well plate and all wells were subsequently sealed with a plastic adhesive to prevent evaporation and maintained at 37°C in a water bath until imaging.

Muscle Phantom—fresh lean bovine muscle (1.5 g) was placed in 50 mL dilutions (2, 1, 0.5, 0.25, 0.125, and 0 mM) of Gd-DPTA in NS for 10 hours with gentle agitation at 4°C. Dilutions were removed and replaced for an additional 2 hours of incubation with gentle agitation at 4°C. Muscle was removed and placed in a 24-well plate containing a fresh, corresponding dilution and brought to 37°C in a water bath prior to imaging.

During phantom imaging experiments at 37°C, temperature was maintained by placing the phantom on an isothermal pad (Deltaphase®, Braintree Scientific, Inc., Braintree, MA).

Cell Culture

The C6 cell line was established from a glioma generated by intravenous exposure of random-bred Wistar-Furth rats to *N,N'*-nitrosomethylurea (45) and is a well-recognized model of glioma in rats. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Gaithersburg, MD) with 5% fetal bovine serum and 100 µg/mL penicillin/streptomycin and 50 µg/mL gentamycin in a humidified incubator at 37°C in 5% CO₂.

Animal Procedures

All animal procedures were approved by the University of Washington Internal Animal Care and Use Committee prior to study initiation. Twelve adult male Wistar rats (Charles River Laboratories, Wilmington, MA) were used in this study – six healthy rats and six rats serially imaged on post-op day (POD) 14 (baseline) and POD 18 (follow-up) after intracranial inoculation with C6 cells. Stereotactic intracranial implantation of 10⁶ C6 cells suspended in 10 µL of DMEM was consistent with previously described procedures (46).

For imaging experiments, animals were induced in an anesthesia chamber with 5% isoflurane mixed with oxygen and subsequently maintained on 2% isoflurane mixed with oxygen via nose cone inhalation. Immediately prior to imaging, a 22 Gauge angiocatheter (Becton-Dickinson, Sandy, Utah) was inserted into the tail vein. The catheter was attached to a small bore bifurcated extension (Smiths Medical, Dublin, OH) containing a dilution of Gd-DPTA in one arm and a NS flush in the other. The catheter setup was maintained with a saline lock until dynamic imaging. During catheter insertion and imaging, animals were placed on an isothermal pad (Deltaphase®) to maintain core body temperature.

On the final day of imaging, animals were anesthetized with 2 mL/kg Beuthanasia-D and then euthanized by intravascular perfusion of the animal with 4% PFA via an open-chest, left cardiac ventricle puncture approach. Brains were removed intact and underwent a secondary fixation in 4% PFA for 24 hours before being placed in 70% ethanol.

MRI Acquisition

All imaging experiments were performed on a 3T whole-body clinical scanner (Philips Achieva, Philips Medical Systems, Best, Netherlands). A transmit/receive (T/R) head coil (Philips Medical Systems) was used for RF transmission and reception during phantom imaging. During animal imaging, a dual coil approach was utilized as previously described (46) that combined the T/R head coil for transmit and a custom-built combined solenoid-

surface coil (34) for RF reception. The combined solenoid-surface coil required that the animal was placed orthogonal to the scanner gantry.

T₁ Phantom Measurements—Images from an inversion recovery 2D spin echo (SE) sequence (TR/TE=6000/10 ms) with ETL=13 and 16 intervals (50, 75, 100, 150, 200, 300, 400, 600, 800, 1000, 1200, 1600, 2000, 3000, 4000, and 5000 ms) were used to measure T_1 . Additional parameters included field-of-view (FOV)=140×140 mm², matrix=140×130, acquisition resolution=1.00×1.08×2.00 mm³ (zero-interpolated to 0.49×0.49×2.00 mm³), and number of excitations (NEX)=2.

T₂ Phantom Measurements: Images from a 2D SE sequence (TR=3000 ms, $\alpha=90^\circ$) with ETL=32 and TE=30, 45, 60, ..., 495 ms were used to calculate T_2 . Additional parameters included FOV=140×140 mm², matrix=140×140, acquisition resolution 1.00×1.00×2.00 mm³ (zero-interpolated to 0.97×0.97×2.00 mm³), and NEX=2.

Dynamic MRI: Images were acquired in the coronal plane relative to the animal from a 3D spoiled gradient echo (SPGR) sequence (TR/TE=12.3/4.5 ms, $\alpha=18^\circ$) with temporal resolution of 3.47 s, NEX=1, FOV=24×24×8.25 mm³, and matrix=64×64×5.5 for an acquisition resolution of 0.38×0.38×1.5 mm³ (zero-interpolated to 0.19×0.19×0.75 mm³, 11 slices). Coincident with the start of the fourth dynamic scan, Gd-DPTA was manually injected at 50 μ L/s followed by a 250 μ L injection of NS at 50 μ L/s. Control animals were injected with either 0.1 ($N=3$) or 0.2 ($N=3$) mmol/kg (0.084 and 0.167 M/L, respectively) of Gd-DPTA. All tumor-bearing animals were injected with 0.2 mmol/kg of Gd-DPTA. Total time for injection of Gd-DPTA and the NS bolus for all animals was <20 seconds. Pre-contrast R_1 maps and post-contrast R_1 maps obtained 5 minutes after CA injection were acquired using the variable flip angle (VFA) method (47) with a 3D SPGR sequence (TR/TE=4.6/20 ms) utilizing $\alpha=4^\circ$ (NEX=3), $\alpha=10^\circ$ (NEX=1), $\alpha=20^\circ$ (NEX=2), and $\alpha=30^\circ$ (NEX=3) to provide an identical multi-slice acquisition and voxel resolution as the dynamic sequence to minimize registration errors. Scan time was 33.8, 16.9, 22.6, and 33.8 s, respectively. VFA data were acquired with variable NEX to provide similar SNR across each flip angle as previously described (46). Pre-contrast B_1 maps were acquired to correct for field heterogeneities using the actual flip angle imaging method (48) with the following parameters: TR₁/TR₂/TE=25/125/6.7 ms and $\alpha=60^\circ$. 3D B_1 maps were acquired with a NEX=1, identical resolution as the dynamic scan, and a scan time of 42.9 s.

Tumor Identification: Fast bound-pool fraction imaging was utilized for high spatial resolution tumor identification as previously described (46). Total scan time to produce f maps was 45.5 minutes.

Image Processing

Phantoms: T_1 and T_2 measurements were obtained from region-of-interest (ROI) data. For each Gd-DPTA concentration ([Gd]), T_1 data were fit to $SI(T_1) = \rho_0(1 - 2e^{-T_1/T_1})$, where SI =signal intensity, ρ_0 =spin density, and T_1 is inversion time. R_1^C was subsequently measured using a linear least-squares fit to the coordinates ([Gd], $1/T_1$ [Gd]). T_2 data were fit

to $SI(T_E) = \rho_0 e^{-T_E/T_2}$, where T_E is time to echo. R_2^C was subsequently determined using a linear least-squares fit to the coordinates ($[Gd]$, $1/T_2[Gd]$).

Animals: Pre- and post-contrast R_1 maps ($R_1=1/T_1$) were reconstructed from the corresponding VFA data using a least-squares linear fit to the signal intensities (S) transformed into the coordinates ($S(\alpha)/\sin\alpha$, $S(\alpha)/\tan\alpha$) (47). Voxel-based $[Gd]$ maps were determined from:

$$\frac{1}{T_1^{\text{post}}} = \frac{1}{T_1^{\text{pre}}} + R_1^C[Gd],$$

where $\frac{1}{T_1^{\text{pre}}}$ and $\frac{1}{T_1^{\text{post}}}$ are the R_1 maps derived from VFA data before and after CA, respectively, and R_1^C is the relaxivity of the CA at 37°C (16,38,39).

Mean values for the rate of CA infusion ($\overline{K_{in}}$), CA elimination ($\overline{K_e}$), and a scaling factor ($\overline{C_p}(20.8)$) were obtained by averaging parameter values after fitting individual curves in the control animals receiving 0.2 mmol/kg of CA. Utilizing $K_m^{\text{trans}}=0.1 \text{ min}^{-1}$ and the mean longitudinal relaxation rate of muscle ($\overline{R_{1m}}$) determined by the average value from all control animals, voxel-based k^{vasc} maps in tumor-bearing animals were subsequently computed by the following two steps: *Step 1* – using Eq. 8, K_{in} and K_e were determined for each animal using the mean SI from ROIs within the temporalis muscle and constraining the fit to a standardized plasma concentration curve ($\overline{C_p}$) defined by $\overline{K_{in}}$ and $\overline{K_e}$ and scaled to $\overline{C_p}(20.8)$; *Step 2* – k^{vasc} maps were constructed from a voxel-based fit of dynamic MR images using Eqs. 6 and 7, where $S(0)$ was the median value from the images prior to CA arrival. B_1 maps were used to correct the voxel-based excitation flip angles for both the VFA and dynamic MRI datasets (Fig. 2).

Tumors were manually outlined using $[Gd]$ maps. Tumor sub-regions were also manually outlined based on apparent visual incongruences between k^{vasc} and $[Gd]$ maps. All image processing was performed using in-house written Matlab (The Mathworks, Natick, MA) and C/C++ language software.

Histology—After fixation, brains were embedded in paraffin, sectioned (5 μm thick), and stained with hematoxylin-eosin. Slides were scanned using an Olympus VS110 virtual microscopy system (Olympus, Center Valley, PA) for display on NDP.view (v2.5.14).

Statistical Analysis—For continuous variables, the mean and standard deviation (SD) was calculated. The student's independent t -test assuming equal or unequal variance based on Levene's test was used to compare mean values between groups. Pearson's correlation coefficient (r) was used to identify associations between parameters. Statistical analyses were performed with SPSS for Windows (Version 12.0, SPSS, Chicago, IL). Statistical significance was defined as $P<0.05$.

Results

Measurement of Gd-DPTA R_1^C and R_2^C

For Gd-DPTA in NS at 37°C, $R_1^C=3.57\text{s}^{-1}$ and $R_2^C=3.95\text{s}^{-1}$ (Supporting Fig. S1). For Gd-DPTA in muscle at 37°C, $R_1^C=3.61\text{s}^{-1}$ and $R_2^C=5.34\text{s}^{-1}$ (Fig. 3).

Determination of Standardized Plasma [Gd] Curve for the Muscle-Based RRM

The signal intensity in the SSS prior to infusion of CA was variable (Fig. 4A–F). The normalized signal intensity (NSI) in the SSS during infusion of either 0.1 and 0.2 mmol/kg of Gd-DPTA was also variable (Fig. 4G–H). In contrast, NSI from the temporalis muscle segregated based on dosage of Gd-DPTA (Fig. 4I–J). Of note, the NSI in the SSS plateaued at $t=20.8$ s whereas the NSI in muscle continued to increase (Fig. 4G and 4I). As such, seven data points (i.e., $N=7$ for Eq. 8) were used for all subsequent animal-derived curve fitting to represent the period of continuous infusion.

The mean ($\pm$ SD) R_1 for muscle from all six control animals was $\overline{R_{1m}}=0.63 \pm 0.03\text{s}^{-1}$ and was used for all subsequent calculations. Determining K_{in} and K_e solely from muscle data yielded strong fits to muscle data, but the reconstructed plasma curve (C_p) based on these values did not correspond with the general shape of the observed variations in NSI from plasma during the continuous infusion of Gd-DPTA (Fig. 5). To provide a $C_p(t)$ input for the constrained fitting described in Eq. 8, $C_p(t)$ was estimated by utilizing Eq. 4, setting $K_m^{\text{trans}}=0.1 \text{ min}^{-1}$, and assuming a linear increase in plasma [Gd] to approximate $C_p(t)$ at $t=20.8$ followed by scaling the NSI from the SSS accordingly. The K_{in} and K_e values produced from this approach provided plasma curves consistent with observed increases in NSI (Fig. 6A–F). There was a significant difference in the ratio K_{in}/K_e between animals given a full vs. low dose (3.41 ± 0.26 vs. 2.14 ± 0.26 mM, respectively; $P=0.007$), which was consistent with the proposed model.

A NSI from the SSS for the constrained fit of muscle data may not be reliably detected in all tumor-bearing animals, particularly when large tumors occur. Accordingly, a standard plasma curve ($\overline{C_p}$) based on the mean K_{in} ($25.1 \pm 8.8 \text{ mM/min}^{-1}$) and K_e ($7.3 \pm 2.1 \text{ min}^{-1}$) and scaled to the mean value of $C_p(20.8)=3.10 \pm 0.51 \text{ mM}$ determined from the 0.2 mmol/kg dosage group was generated. Differences in plasma curves and fitted data for both muscle and plasma are depicted in Figure 6 (G–I). There was not a significant difference in determination of K_{in}/K_e using the standard curve compared to individual curves (3.25 ± 0.18 vs. 3.41 ± 0.26 mM, respectively; $P=0.49$). Subsequently, these parameters ($\overline{K_{in}}=25.1 \text{ mM/min}^{-1}$, $\overline{K_e}=7.3 \text{ min}^{-1}$, and scaling factor of $\overline{C_p}(20.8)=3.10 \text{ mM}$) were used during the constrained fit of muscle data in tumor-bearing animals during voxel-based determination of k^{vasc} .

Tumor Imaging

Tumor phenotypes on f maps were consistent with morphology on histology (Figs. 7 and 8). At baseline, the intratumor median [Gd] and the median k^{vasc} ranged from 0.04–0.15 mM and 0.04–0.21 min^{-1} , respectively. On follow-up, the intratumor median [Gd] and the

median k^{vasc} ranged from 0.06–0.30 mM and 0.06–0.26 min⁻¹, respectively. There was a strong correlation in median values between baseline and follow-up for intratumor [Gd] ($r=0.94$, $P=0.0054$) and intratumor k^{vasc} ($r=0.94$, $P=0.0052$). At baseline, voxel-based correlations between intratumor [Gd] and intratumor k^{vasc} were present ($0.26 < r < 0.85$, $P < 0.001$). At follow-up, voxel-based correlations between intratumor [Gd] and intratumor k^{vasc} were also present ($0.45 < r < 0.82$, $P < 0.001$; Figs. 9 and 10). However, sub-regions within each tumor at follow-up demonstrated an absence of association between intratumor [Gd] and intratumor k^{vasc} ($N=5$; $-0.08 < r < 0.22$, $P > 0.05$; Fig. 9A and C, Fig. 10A–C) or the association became inverted ($N=1$; $r = -0.28$, $P=0.001$; Fig 9B). These latter findings indicated the presence of differences within tumors between locations of blood-brain barrier disruption and the subsequent pooling of CA. A consistent underlying microscopic tissue appearance to explain the discordance was not present (Supporting Figs. S2 and S3).

Discussion

In this study, a model based on the continuous infusion of a CA and the accompanying temporal T_1 -weighted variations in contrast enhancement was implemented for the serial evaluation of tumor-associated blood-brain barrier permeability changes in an animal model of GBM. In 1991, Brix et al. proposed the continuous infusion model to describe the kinetics of contrast enhancement in the brain (17). The model utilized data points during and after the infusion as part of a non-linear fitting of three-parameters: k_{21} , the rate constant for transfer of contrast from the EES back into the central compartment; k_{el} the rate constant for elimination of contrast from the central compartment; and a third variable, A , that was dependent on the tissue, the sequence parameters, and the infusion rate (17). Subsequent application of the continuous infusion technique in the brain; however, was largely limited to a single publication (49). Wider implementation may have been limited, at least in part, due to a study by Tofts and Berkowitz that compared continuous-infusion to bolus-infusion models and found the latter was more efficient and easier to administer (50). A critical difference between the methodology described herein and previous applications of the continuous infusion model was infusion time. The infusion time utilized in the present study (< 20 s) was substantially shorter than that used by Brix et al. (4 min; (17)), Kenney et al. (20 min; (49)), and Tofts and Berkowitz (50 min; (50)). Longer continuous infusion times lead to reduced tissue enhancement compared to a bolus infusion (50) and permit the occurrence of inter-voxel diffusion that may confound results (51). In the present study, these potential effects were mitigated by a relatively short infusion time and rapid image acquisition during dynamic scanning that yielded seven data points for model fitting of only a single parameter. This approach produced longitudinal parametric maps of permeability that identified a range of values within and between tumors that included differences and similarities between increased blood-brain permeability and CA accumulation.

The implementation of a RRM as a surrogate for a vascular-based AIF has several advantages. First, the use of a reference point from extracranial healthy tissue (i.e., muscle) avoids tumor-associated effects (e.g. increased intracranial pressure, edema, etc.) that may confound parameter determination during the study of intracranial tumors. In addition, the use of a RRM may afford the opportunity to more directly compare results derived from different methods that yield incongruent results. In a subcutaneous model of

adenocarcinoma, Yankeelov et al. used a three-parameter fit that included K_m^{trans} and found $K_m^{\text{trans}}=0.27 \text{ min}^{-1}$ with an intratumor $K^{\text{trans}}=0.13 \text{ min}^{-1}$ (43). In a similar study that used a subcutaneous model of lung carcinoma, Yankeelov et al. fixed $K_m^{\text{trans}}=0.1 \text{ min}^{-1}$ and implemented a two-parameter fit that reported an intratumor $K^{\text{trans}}=0.35 \text{ min}^{-1}$ (44). Although seemingly different, these studies actually yield comparable K^{trans} values once scaled relative to muscle permeability. In the present study, a fixed value for healthy muscle permeability ($K_m^{\text{trans}}=0.1 \text{ min}^{-1}$) was used and the subsequent intratumor k^{vasc} values reported herein more strongly reflect the values in the study described by Yankeelov et al. that also used a $K_m^{\text{trans}}=0.1 \text{ min}^{-1}$. Although the use of fixed K_m^{trans} re-introduced the assumption that healthy muscle permeability is consistent between animals with intracranial tumors, this seemed a reasonable supposition to gain the advantage of preserving model stability and introducing a reference scale for comparison of k^{vasc} values. An additional benefit of the RRM is that a standard intravascular distribution of contrast agent is not assumed. Although Ng et al. found that pharmacokinetic parameters are not substantially altered using a common AIF vs. individually measured AIFs (24), the common AIF employed by Ng et al. was derived from the mean of the individual AIFs. Furthermore, different types of AIFs have been described and the selection of different functions has been shown to effect parameter determination (52). Accordingly, the effects on parameter determination of using a standardized AIF across multiple studies that may vary in methodology remains unclear. Therefore, a strength of RRM is the opportunity to account for the inter-animal variability inherent to contrast infusions due to heart rate and contrast delivery, while concomitantly optimizing the modeling of the AIF.

Spatial and temporal resolution are critical aspects of dynamic imaging. Utilizing an accelerated temporal resolution of 4.97 s in patients with abdominal or pelvic masses, Parker et al. found the AIF associated with a bolus injection as a first-pass peak followed by a recirculation peak and then a washout period (53), which was distinctly different from the commonly used monotonically bi-exponential decay AIF (54). Differences between the modeled AIF were subsequently shown to cause statistically significant differences in the determination of K^{trans} , v_p , and v_e (53). In this study, the high temporal resolution of 3.47 s enabled sufficient acquisition of data points during a continuous, albeit rapid, infusion of contrast for determination of a single parameter, k^{vasc} . Importantly, the use of a customized coil on a clinical scanner enabled a high temporal resolution without compromise of spatial resolution (acquisition resolution of $0.38 \times 0.38 \times 1.5 \text{ mm}^3$) or image quality. Spatial resolution of the rat brain during dynamic imaging on small bore animal magnets (range: 6.3–9.4T) using a similar temporal resolution (range: 2–4.8 s) has spanned from $0.27 \times 0.27 \times 1.5$ to $0.33 \times 0.33 \times 2.0 \text{ mm}^3$ (27,29,36,55). The similarity in acquisition parameters without sacrificing apparent image quality between the study described herein and investigations conducted at higher field-strengths supports the assertion by Pillai et al. that specialized coils may allow greater accessibility to animal-based MRI studies at centers without animal scanners (33).

Several elements of the study design merit consideration. First, the specific approach for determining the relaxivities of the CA in muscle has not been previously described. The

measurement is dependent on the passive diffusion of CA throughout the EES of muscle. This assumption is supported by early studies of the blood-brain barrier where brain tissue and muscle were incubated with various substances to measure permeability and muscle was utilized as the reference tissue for maximum diffusion (56). As such, alternative strategies to determine R_1^C and R_2^C in muscle may yield different values and alter the subsequent estimation of k^{vasc} . Second, the proposed continuous infusion approach was not compared to an existing bolus-based methodology. Identifying a specific parameter to evaluate potential associations between methods is limited by the underlying premise of the current model that the individual effects of the abnormal tumor-associated MVP (i.e., K^{trans} , v_p , and v_e) are inseparable and collectively represented by k^{vasc} . In addition, simulating bolus-based methods requires assumptions that conflict with the current model. Specifically, commonly used bolus-based methods such as the modified Kety-Tofts model (a three-parameter model that assumes bidirectional transfer of CA) (39):

$$C_t(t) = v_p C_p(t) + K^{\text{trans}} \int_0^t C_p(t') e^{-\frac{K^{\text{trans}}}{v_e}(t-t')} dt'$$

or the Patlak model (a two-parameter model where efflux of CA from the EES back into the vascular space is assumed negligible) (57):

$$C_t(t) = v_p C_p(t) + K^{\text{trans}} \int_0^t C_p(t') dt'$$

define v_p based on the first image acquired in correspondence with maximum signal intensity on the AIF (AIF_{max}). However, determination of v_p is affected by bolus delivery timing and dynamic acquisition time in conjunction with both vascular structure and K^{trans} . Accounting for effects prior to AIF_{max} affects the determination of both K^{trans} and v_p (21). As such, it is difficult to make simulation-based comparisons as the continuous infusion-based approach models effects throughout the infusion process, while commonly used bolus-based methods generally disregard properties prior to AIF_{max} and model the subsequent effects. And finally, histology was solely utilized for evaluating tumor morphology rather than a broader immunohistochemistry analysis to identify associations between k^{vasc} and specific tissue properties. Comparison of dynamic MRI parameters to histology requires the reduction of a three-dimensional active process to a two-dimensional static state. Challenges associated with this reduction in GBM are evidenced by a previously reported absence of an association between the Gd-DPTA uptake rate constant and microvessel density (27). As such, comparisons between histology and dynamic MRI in GBM have been largely divided into two categories: 1) associations with tumor grade (19,58); and 2) utilization of focal histology findings for comparison to tumor-based dynamic parameters (32,59,60). Future studies may benefit from a complete multi-slice histologic analysis of tumors utilizing an array of immunohistochemistry to produce three-dimensional multi-parameter histology maps to better understand tissue properties that influence dynamic MRI parameters.

Conclusions

High spatial and temporal resolution coupled with a continuous infusion-based model of the pharmacokinetics of dynamic T_1 -weighted contrast enhancement provided a quantitative measure of the effects associated with intracranial tumor-associated MVP. The muscle-based RRM served as an effective surrogate for the AIF enabling serial monitoring of tumor-associated vascular changes while obviating potential interference of tumor growth on the intracranial vessels commonly used for the AIF. Finally, the implementation of the proposed methodology on a 3T clinical scanner may broaden utilization of animal studies, while also facilitating the translation of the technique to investigations of brain tumors in human patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Two-compartment open model between the vascular space (C_p) and tumor tissue (C_t). A CA is infused at a constant rate (K_{in}) over a fixed interval. K_e is the elimination of CA from the plasma volume either through excretion or distribution into tissues. The diffusion of CA into tumor tissue is a function of permeability, K^{trans} .

Figure 2.

Images required for the construction of k^{vasc} maps. B_1 maps provide a voxel-based correction of flip angles associated with pre-contrast variable flip angle (VFA) data points during the construction of R_1 maps. R_1 maps are then used with dynamic images and B_1 maps (for voxel-based flip angle correction associated with Eq. 6) to synthesize k^{vasc} maps.

Figure 3.

Images of the phantom and associated fitted data for determination of R_1^C (A) and R_2^C (B) of Gd-DPTA in muscle at 37°C. Each panel contains an image of the phantom (left), plots of signal intensity as time to IR (R_1^C) or TE (R_2^C) was varied across different concentrations of Gd-DPTA (center), and regression models for final determination of R_1^C and R_2^C (right).

Figure 4. Measuring the effects of Gd-DPTA in the SSS and muscle in rats without tumors. Pre-contrast coronal images (A–F) of the rat brain displayed at a uniform window and level setting. The signal in the SSS (A–F, white arrow) is variable in all six animals ranging from absent to hyperintense. In G, the normalized signal intensity of the SSS after administration of Gd-DPTA (lower case letters correspond to animals A–F; curve (a) and (*) represent different slices from the same animal). In H, the mean ($\pm$ SD) normalized signal intensity in the SSS at each dose is represented. In I, the normalized signal intensity from muscle

adjacent to the calvarium is shown (lower case letters correspond to animals A–F). In J, the mean ($\pm$ SD) normalized signal intensity from muscle for each dose of Gd-DPTA is depicted.

Figure 5.

Determination of K_{in} and K_e using only muscle data. Although the overall fit (C_t , gray solid line) to the muscle data (gray squares) is strong, the shape of the theoretical blood curve (C_p , black solid line) applying the determined K_{in} and K_e to Eq. 5 does not match well to the observed blood signal (black circles).

Figure 6.

Solid lines (A–F) represent the fitted curves for muscle (gray) and blood (black) for each determination of K_{in} and K_e from the full-dose (A–C) and half-dose (D–F) animals using Eq. 8 to constrain the fit of muscle data (gray squares) to the general shape of the blood data (black circles). Note that the fit to muscle data is strong while providing K_{in} and K_e values that yield a blood curve consistent with observed imaging data. In G–I, dashed lines have been added to the plots from the full-dose animals (A–C) that represent the fitted curves for muscle (gray) and blood (black) using the standardized blood data (black diamonds) derived from the average values of K_{in} and K_e individually measured from the full-dose animals to constrain the fit of muscle data.

Figure 7. Serial parametric maps from post-op day (POD) 14 and POD 18 with corresponding histology from POD 18 of rat brain tumors. On f maps the tumors (white arrowheads; A–C) appeared hypointense compared to surrounding gray matter. White matter structures (e.g., see A: anterior limb, anterior commissure, AA; corpus callosum, CC) were hyperintense. Tumor phenotypes were consistent with the morphology on histology (magnifications shown in insets). Tumors (A–C) were well-circumscribed bulky lesions with smaller projections extending along the injection track or laterally along the inferior aspect of the corpus

callosum (A and C). In C, there was a dural growth of the tumor (blue arrowheads) that was lost during histological processing. Compared to pre-contrast R_1 maps, post-contrast R_1 maps identified locations of contrast accumulation. In A, a circumferential increase in k^{vasc} surrounding low k^{vasc} values (white arrow) had a corresponding area of diffuse contrast accumulation on the post-contrast R_1 map (black arrow). In B, the initial accumulation of contrast (POD 14, black arrow) corresponded to increased k^{vasc} values (POD 14, white arrow). By POD 18 there was a strong ring-like distribution of increased k^{vasc} values (white arrow) with a central region of contrast accumulation (black arrow). In C, there was an initial speckled increase in k^{vasc} values (POD 14, white arrow) with an apparent corresponding regional accumulation of contrast (POD 14, black arrow). By POD 18, there was a diffuse intratumor increase in k^{vasc} (white arrow) associated with a diffuse accumulation of contrast (black arrow). Window and level for each parametric map are identical in all corresponding images for Figures 7 and 8. H&E = hematoxylin and eosin stain. The black bar at the bottom right corner of each histology inset represents a length of 1 mm.

Figure 8.

Serial parametric maps from post-op day (POD) 14 and POD 18 with corresponding histology from POD 18 of rat brain tumors. On f maps the tumors (white arrowheads; A–C) appeared hypointense compared to surrounding gray matter. White matter structures (e.g., see A: anterior limb, anterior commissure, AA; corpus callosum, CC) were hyperintense. Tumor phenotypes were consistent with the morphology on histology (magnifications shown in insets). Tumors either grew outward from along the injection track (A) or were well-circumscribed bulky lesions (B and C). In A, the strongest increase in k^{vasc} (white arrow)

was disparate from the accumulation of contrast (black arrow), which was inferiorly located. By POD 18, there were two distinct elevations in k^{vasc} (white arrows), while the general distribution of contrast accumulation remained similar (black arrow). In B, the accumulation of contrast (POD 18, black arrow) was derived largely from an inferior-based vascular network as evidenced by the k^{vasc} map (white arrow). In C, there was relatively minor change in the overall accumulation of contrast between time points (black arrows), which was mirrored by the relatively stable k^{vasc} values and distribution (white arrows). Window and level for each parametric map are identical in all corresponding images for Figures 7 and 8. H&E = hematoxylin and eosin stain. The black bar in the bottom right corner of each histology inset represents a length of 1 mm.

Figure 9.

Parametric maps from each animal at POD 18 for k^{vasc} and [Gd] that correspond to images and histology from animals shown in Figure 7. The tumor was outlined in blue based on appearance on the [Gd] map, while a sub-region within each tumor was outlined in yellow. Scatter plots are shown for the tumor (blue + blue/yellow dots) and each sub-region (blue/yellow dots). The solid blue line and solid yellow line correspond to the best linear fit through the tumor and the sub-region, respectively. The associated Pearson's r -value and P -value correspond to the colors of the solid lines.

Figure 10.

Parametric maps from each animal at POD 18 for k^{vasc} and [Gd] that correspond to images and histology from animals shown in Figure 8. The tumor was outlined in blue based on appearance on the [Gd] map, while a sub-region within each tumor was outlined in yellow. Scatter plots are shown for the tumor (blue + blue/yellow dots) and each sub-region (blue/yellow dots). The solid blue line and solid yellow line correspond to the best linear fit through the tumor and the sub-region, respectively. The associated Pearson's r -value and P -value correspond to the colors of the solid lines.