Original Research

Assessment of Brain Tumor Angiogenesis Inhibitors Using Perfusion Magnetic Resonance Imaging: Quality and Analysis Results of a Phase I Trial

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Purpose: To determine thresholds of quality for a T2*-weighted perfusion magnetic resonance imaging (MRI) study and evaluate the effects of an angiogenesis inhibitor on relative blood flow and volume changes in brain tumor patients in a multi-institution setting.

Materials and Methods: A total of 36 volunteers from four participating institutions with clinically diagnosed malignant gliomas were studied using perfusion MRI protocols. These included a baseline study and follow-up studies every eight weeks to evaluate the effect of an anti-angiogenic agent on tumor perfusion. Quality tests were performed on the perfusion imaging data by defining statistical thresholds of acceptance. Region of interest (ROI) analysis was performed on tumors and kinetic parameters were normalized with respect to normal tissue.

Results: Statistical thresholds for goodness of the gamma variate fit, T2* recovery, and mean signal full-width half-minimum (FWHMin) were computed for our data sets with a 99% one-sided confidence interval; these were 6.91%, 79.48%, and 23.35 seconds, respectively. Decreases in-blood volume and flow measurements were observed in patients with documented clinical response.

Conclusion: Malignant brain tumors have altered perfusion parameters that may be used to understand and monitor neovascularization. This permits non-invasive assessment of the efficacy of angiogenesis inhibiting drugs.

Key Words: brain tumors; angiogenesis; dynamic susceptibility contrast enhanced MRI (DSC-MRI); cilengitide; cerebral blood flow (CBF); cerebral blood volume (CBV)
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SEVERAL MAGNETIC RESONANCE imaging (MRI) techniques have been successfully devised to study brain tumors in a clinical setting ranging from radiologic diagnosis, description of tumor characteristics, evaluation of size and volume, and determination of biochemical information. The chief advantage of using MRI over other imaging modalities is the excellent soft tissue contrast it affords coupled with very good resolution and sensitivity. This work describes an attempt to acquire and analyze perfusion MRI data from patients with primary brain tumors that were enrolled in an early phase clinical trial of the anti-angiogenic compound, EMD 121974 (cilengitide). This was done to establish statistical quality metrics and evaluate the effects of cilengitide therapy on relative cerebral blood flow (CBF) and cerebral blood volume (CBV) changes in these patients.

Angiogenesis is an essential component of tumor progression in which neovasculature nourishes growing tumors and facilitates tumor expansion beyond 2 mm^3 (1–3). Malignant gliomas are among the best vascularized tumors in humans (4,5). Angiogenesis inhibiting agents, like EMD 121974 used in this study, are particularly promising for brain tumors because these tumors have marked neovascularization, significant molecular alterations producing an angiogenic and invasive phenotype, and a poor clinical outcome that correlates with an angiogenic phenotype (6,7).

Perfusion is the steady state delivery of blood to tissue parenchyma through the capillaries (8). Perfusion

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MRI may be used to evaluate kinetic parameters of blood in normal and pathologic conditions of the central nervous system. Applications of perfusion MRI like dynamic susceptibility contrast-enhanced MRI (DSC-MRI) have shown that bolus administration and tracking of a paramagnetic contrast medium can be used to quantify regional blood flow and volume (9–13).

Contrast-enhanced MRI has been used to evaluate tumors by several groups primarily to characterize tumor vascularization and flow properties and correlate these with histologic grade (14–17). Daldrup et al (14) have shown a highly positive correlation between tumor permeability to macromolecular contrast medium and tumor grade. MR-derived relative CBV maps have also correlated with histologic measures of microvessel density from surgical tissue in the evaluation of tumor angiogenesis (15). Sugahara et al (18) demonstrated a relationship in astrocytic gliomas between the maximum CBV and histologic vascularities that correlated with tumor grade. Others have used T2*-weighted MRI perfusion sequences for generating blood volume maps to improve the accuracy of stereotactic biopsies. Knopp et al (19) used areas of perfusion abnormality to aid targeting of biopsies in patients with astrocytomas. T2*-weighted perfusion methods have also been shown to be effective in computing permeability of high and low grade glial neoplasms while being consistent with earlier results reported using T1-weighted methods (20).

Maximum signal drop and area under the signal-time curve have been monitored to provide information about blood kinetics (21). The blood volume of glioblastoma multiforme (GBM) has been evaluated both qualitatively and quantitatively using dynamic perfusion-weighted imaging and it was shown that double-echo imaging may be more suitable for analysis of blood volume in GBM (22). Other attempts have been made to quantify fractional volumes of different glioma compartments as well as vessel permeability and CBF using T1-weighted dynamic MRI towards pharmacokinetic characterization of gliomas (23). Variations in the recirculation characteristics of a contrast agent bolus have been related to tumor grade in gliomas. Abnormalities in contrast agent recirculation (24) provide independent information concerning the microcirculation in imaging studies of angiogenesis and may be useful in the assessment of trials of anti-angiogenic therapies like the one attempted in this study. DSC-MRI techniques have been tested as surrogate markers of tumor response to anti-angiogenic therapy in xenograft models of human gliomas (14,25) and reductions in relative CBV correlated well with tumor response.

Functional perfusion imaging allows for the evaluation of the whole tumor and not selected areas biased by biopsy samples. However, the use of imaging based surrogate markers for the assessment of the angiogenic process is strongly dependent on their test-retest reproducibility. Jackson et al (26) formally tested the reproducibility of T2* blood volume and vascular tortuosity maps in cerebral gliomas and concluded that measurement of relative blood volume in consecutive studies is statistically capable of reliably detecting changes in excess of 15% in between group studies and 25% in individual patients.

In this report, we evaluated the acquisition and analysis of DSC-MRI in a multi-institutional setting. We will report on the statistical analysis of DSC-MRI data sets from a clinical trial and our attempt to define thresholds for quality and reliability. For those studies that meet the threshold measures, we have analyzed for perfusion parameters in a large population of recurrent malignant gliomas. This represents one of the first efforts to apply the principles of DSC-MRI to the clinical issue of early phase assessment of anti-angiogenic agents.

MATERIALS AND METHODS

EMD 121974 (Cilengitide): Angiogenesis Inhibitor

EMD 121974 is a synthetic pentapeptide supplied in solution form for parenteral administration (Merck KgaA, Darmstadt, Germany). It is supplied by the Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), as an isotonic solution containing 450 mg of lyophilized EMD 121974 dissolved in 30 mL of sodium chloride and water for injection (at a concentration of 15 mg/mL). This angiogenesis inhibitor is a potent and selective $\alpha v\beta 3$ and $\alpha v\beta 5$ vitronectin receptor antagonist.

Patients who were eligible to receive the drug were enrolled in NABTT 9911: a Phase I study of EMD 121974 for patients with recurrent malignant glioma, conducted by the NCI Central Nervous System Consortium, New Approaches to Brain Tumor Therapy (NABTT). The clinical trial used a standard phase I dose escalation design. EMD 121974, with a starting dose of 120 mg/m^2, was administered twice a week, intravenously, over one hour. The dose of the drug was escalated in a stepwise fashion. The first five dose levels were 120, 240, 360, 480, and 600 mg/m^2. The protocol was subsequently amended to allow for further dose escalations leading to dose levels of 1200, 1800, and 2400 mg/m^2.

Perfusion MRI

The perfusion imaging sequences were run on 1.5 T scanners of different manufacturers—GE (Signa 5.7; GE Medical Systems, Milwaukee, WI), Siemens (Magnetom Vision; Siemens Medical Systems, Erlangen, Germany), and Philips (Gyroscan ACS-NT; Philips Medical Systems, Best, The Netherlands)—depending on trial site. A total of 36 patients with clinically diagnosed malignant gliomas were imaged during multiple sessions. These included a baseline scan before first administration of the angiogenesis inhibitor and follow-up perfusion scans performed after every eight weeks of treatment with EMD 121974. Twenty-one patients were imaged multiple times, while the others had only a baseline scan.

There were a total of 72 perfusion MRI studies analyzed during the preliminary phase of this work. Due to the multi-institution setting of this study, the DICOM (digital imaging and communication in medicine) standard was used for image storage, exchange, and query.

The chief objectives of the DICOM standard are to achieve compatibility and improve the reliability and efficiency between imaging acquisition systems and other information systems like picture archiving and communication systems in healthcare environments worldwide.

The gradient recalled echo-planar imaging (EPI) sequence was used for acquiring functional perfusion data. This study used a TR of 1900 msec and a TE of 50 msec, accommodating a 20% variation in both TR and TE to permit machine/trial site specific acquisition settings. A 24 cm × 24 cm field of view was used and 5–10 (depending on the size of the tumor) 6- to 8-mm axial slices passing through the center of the tumor were imaged over 40 to 65 functional time points resulting in a total scan time of just over two minutes. In some instances, the tumors were larger than the volume imaged by five slices; in such cases, a greater number of slices were imaged and then the five contiguous slices with the highest mean blood flow and volume were utilized for interpatient analyses of the hemodynamics.

Spin-echo (SE) postcontrast T1-weighted images were acquired following perfusion imaging for anatomic reference. These images were acquired with a TR of 450 msec and TE of 10 msec. The standard dose of the gadolinium based contrast agent was 0.2 mmol/kg of patient body weight. The contrast agent was injected with a power injector at a flow rate of 4.0 mL/second and an injection delay of 15 seconds.

Perfusion Analysis

Post processing and perfusion analysis was performed using the MedX software (version 3.4.2; Sensor Systems, Sterling, VA) running on a Sun Blade 1000 workstation (Sun Microsystems, Palo Alto, CA) with the Solaris 8 Operating System.

Each of the *N* anatomic slices imaged at 40–65 time points were used to generate a statistical mean image in preparation for temporal plotting of the susceptibility curves. These mean functional images are then inspected for quality, contrast agent induced susceptibility behavior, and artifacts. In agreement with the theoretical models available, five distinct features were observed (8,27):

1. A baseline phase consisting of the signal before contrast agent arrives in the tissue,
2. Arrival of the contrast agent triggering a drop in signal intensity,
3. Maximum signal drop that occurs when the highest concentration of contrast agent is present in the blood vessels,
4. A wash out phase marked by a recovery of the signal, and finally,
5. A postinjection signal that is slightly lower than the preinjection baseline because the concentration of the gadolinium is still sufficient to cause a slight signal drop.

The data sets were prepared for postprocessing by inspection for quality, masking, and generation of parametric maps. Masking of the functional data based on an empirically determined threshold was performed to ensure accurate determination of arterial input function and to avoid curve-fitting of pixels outside the brain. A two-stage automatic algorithm (28) was used for identifying arterial voxels in the DSC-MRI data and constructing the arterial input function (AIF). This method uses multiple "arterial likelihood" metrics to choose the best candidate input function for computing the AIF. Motion correction and spatial filtering were not performed. The arterial and mean tissue curves are computed based on the signal-time curves. The arterial likelihood map was computed based on maximizing P (large peak-height), where P represents the probability. Of the top 45 pixels in the selected arterial likelihood map, the first 20 pixels were excluded as noise and the next 25 were averaged to create the input function. The relative CBV maps are determined though gamma variate fitting (29) of the concentration curves and integration. The relative CBF maps are generated from the amplitude of the residue curve that results from deconvolution of the tissue curve via singular value decomposition. A three-point temporal smoothing is applied before analysis and a constant noise model is assumed. Fitted measures and goodness of fit were computed for each data set.

A three parameter gamma variate model (Eq. [1]) was used for computing the contrast agent concentration curve $C(t)$:

$$C(t) = K\,\tau^{\beta}\,e^{-\tau\alpha} \qquad (1)$$

Here, τ refers to time from bolus arrival, K is a scaling constant, and α and β are additional "rate" parameters. The τ in this expression equals $t–t_0$ where t_0 is the arrival or takeoff time.

T2* recovery was computed by calculating the average signal intensity of the last 20 time points as a percentage of the pre-contrast maximum intensity.

Tumor Region of Interest (ROI) Analysis

Once perfusion analysis was completed, ROI analysis was performed. The ROIs were drawn manually using a simple computer pointing device. Four regions of the brain were analyzed in this study: the hemisphere containing the tumor, the contralateral hemisphere, an ROI encompassing the tumor, and an identical region containing healthy tissue, preferably gray and/or white matter, from the uninvolved hemisphere. The hemispheric regions were outlined on the echo-planar perfusion images. The tumor ROIs were drawn on the T1-weighted post-contrast images. An identical area was outlined in the contralateral area of the brain for baseline comparison and statistical evaluation of kinetic parameters. Care was taken to not include the ventricles and bone in the tumor and contralateral ROIs. These regions were used for analysis of the functional data and to generate relative CBV and CBF maps for each slice through the tumor. Normalized ratios were determined for relative CBV and CBF by taking the mean of the slices divided by the contralateral hemisphere or contralateral ROI.

Table 1
EMD 121974 Multiinstitution Phase I Trial Site Breakdown

Institution	No. of patients	No. of perfusion studies
University of Alabama at Birmingham (UAB)	18	49
Henry Ford Hospital (HFH)	11	16
Massachusetts General Hospital (MGH)	5	5
Emory University (EMU)	2	2

Statistical Analysis

Statistical analysis was performed using SAS (SAS Institute, Cary, NC). A 99% confidence interval was used to determine cutoff thresholds. A one-sample t-test determined the significance of relative CBV and CBF for ROI and hemispheric analyses. A Student's t-test was performed to compare relative CBV and CBF indices of patients with documented clinical responses versus those with progressive disease.

RESULTS

Demographics of the Multi-Institution Trial

The imaging studies analyzed in this report were performed as a component of the NABTT 9911 clinical trial. A total of four institutions were involved in the current phase I trial and are listed in Table 1. Listed in Table 2 are the trial test subjects and their clinical diagnosis.

Data Quality and Reliability

The temporal characteristics of a typical contrast-enhanced perfusion study indicating the MR induced susceptibility drop, the minimum signal intensity time, the signal full-width at half-minimum (FWHMin), and T2* recovery are demonstrated in Fig. 1a. All the data sets were inspected with respect to multiple measures of perfusion reliability. These included the susceptibility characteristics and goodness of the gamma variate fit. Statistical thresholds were computed for these measures. Individual measurements are shown in Fig. 2. The goodness of the gamma variate fit was chosen as a measure of the perfusion analysis efficiency. The FWHMin, minimum signal intensity time and T2* recovery were selected as they reflect the full physical behavior of the MR signal recovery after the susceptibility induced drop.

The 72 perfusion data sets were analyzed for goodness of the gamma variate fit for all pixels by checking the values of α and β. A pixel was identified as a poor fit if α was negative and β was less than 1, where α and β are the parameters in Eq. [1]. Thompson et al (30) showed that these arbitrary distribution parameters determine the shape of the concentration curves. Our data had a mean failure rate of 5.77% (σ = 3.74%). The gamma variate fit failure rates for all the studies are illustrated in Fig. 2. The mean signal FWHMin, T2* recovery, and mean MR-induced susceptibility drop were 19.56 seconds (σ = 12.50 seconds), 82.79% (σ = 10.88%), and 55.30% (σ = 12.71%), respectively. The average susceptibility drops were computed by using pixel information from four randomly chosen pixels, one from each quadrant in the images. These pixels were chosen from healthy tissue, i.e., outside the tumor region being studied.

Using a 99% one-sided confidence interval's upper limit, the cut-off thresholds were determined to be 6.91% for gamma variate fit failure rate, 79.48% for the T2* recovery, and 23.35 seconds for the signal FWHMin. Using such a statistical approach allowed us to minimize the T2* effects during the gamma variate fit. All data sets that passed the statistical thresholds for the three above mentioned parameters had a mean minimum signal intensity time of 36.74 seconds (σ = 7.32 seconds), corresponding to maximum contrast agent concentration. Data sets that did not fall within

Table 2
NABTT 9911 Demographics

Patient no.	Dose level	Number of studies	Test site	Clinical diagnosis
1	1	4	UAB	Glioblastoma multiforme
2	1	2	UAB	Glioblastoma multiforme
3	1	3	UAB	Anaplastic astrocytoma
4	1	6	UAB	Glioblastoma multiforme
5	1	2	UAB	Anaplastic astrocytoma
6	1	2	UAB	Glioblastoma multiforme
7	2	2	UAB	Glioblastoma multiforme
8	2	1	HFH	Anaplastic oligodendroglioma
9	2	3	UAB	Anaplastic astrocytoma
10	2	1	HFH	Anaplastic astrocytoma
11	2	2	UAB	Glioblastoma multiforme
12	2	2	UAB	Glioblastoma multiforme
13	2	3	HFH	Glioblastoma multiforme
14	3	1	HFH	Glioblastoma multiforme
15	3	2	HFH	Glioblastoma multiforme
16	3	5	UAB	Anaplastic astrocytoma
17	3	1	EMU	Glioblastoma multiforme
18	3	5	UAB	Glioblastoma multiforme
19	3	1	UAB	Mixed glioma
20	4	1	EMU	Glioblastoma multiforme
21	4	1	MGH	Glioblastoma multiforme
22	4	1	MGH	Anaplastic astrocytoma
23	4	1	MGH	Anaplastic astrocytoma
24	4	2	UAB	Glioblastoma multiforme
25	4	2	HFH	Glioblastoma multiforme
26	5	1	HFH	Glioblastoma multiforme
27	5	2	UAB	Glioblastoma multiforme
28	5	2	HFH	Anaplastic astrocytoma
29	5	1	MGH	Glioblastoma multiforme
30	5	1	HFH	Glioblastoma multiforme
31	6	2	UAB	Glioblastoma multiforme
32	6	1	HFH	Glioblastoma multiforme
33	6	1	HFH	Glioblastoma multiforme
34	6	2	UAB	Anaplastic astrocytoma
35	6	1	MGH	Mixed glioma
36	8	2	UAB	Glioblastoma multiforme

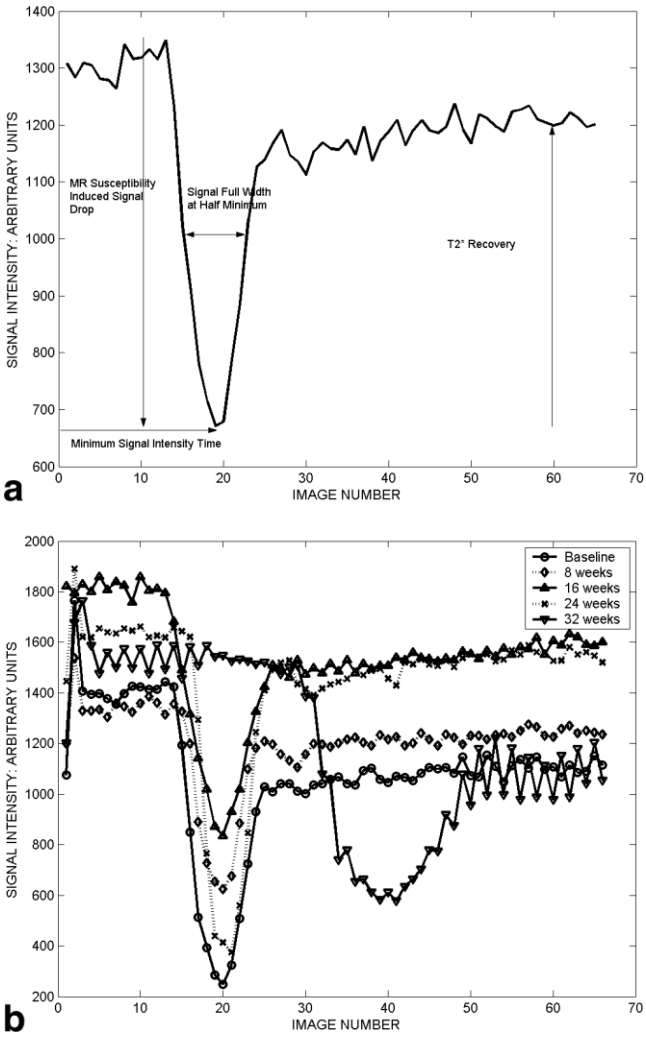

Figure 1. MR signals. The upper graph (**a**) shows a typical functional time course of a contrast-enhanced perfusion study. The lower graph (**b**) shows pixel time courses from five different perfusion data sets for patient 16. The 32-week study of this patient was discarded because of temporal aberration and a high pixel fit failure percentage.

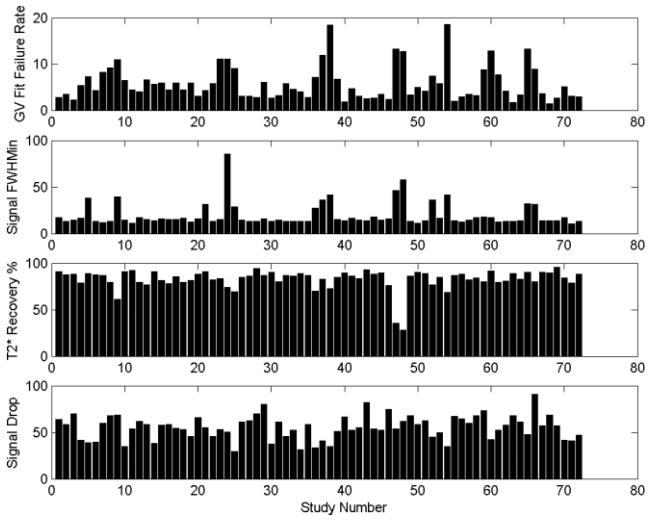

Figure 2. Individual quality measurements for the perfusion data sets. The bar charts represent the four metrics used to statistically standardize the perfusion data sets: the gamma variate fit failure rate, the signal full width at half minimum, the T2* recovery percentage, and MR susceptibility induced signal drop.

the 99% confidence interval for at least one of the three factors were discarded from further post-processing. Fifty-nine of the data sets passed these quality thresholds. These data sets were then considered for ROI analysis provided they did not have any significant motion or other artifacts.

The importance of statistically determining thresholds to limit data is illustrated in the susceptibility curves of five studies from patient 16 shown in Fig. 1b. Notice that while four studies (baseline, 8-week, 16-week, and 24-week) demonstrated a pattern consistent with the expected theoretical behavior, the fifth study (32-week) shows a temporal aberration in its susceptibility characteristics and was consequently discarded for further post-processing. The temporal shift in signal drop in this study suggests a delayed arrival of the bolus caused by discrepancies in the contrast injection procedure. Data exhibiting such procedural irregularities are hence rejected by our data quality inspection process. The gamma variate pixel fit failure percentages and MR signal statistics for these five studies are tabulated in Table 3.

Postprocessing and ROI Analysis

Postprocessing was performed on the 59 data sets that met our defined thresholds for quality. ROI analysis of relative CBF and CBV were performed with emphasis on normalized kinetic ratios. The mean ratios for each of these studies are illustrated in Fig. 3. The data represented by diamonds are values for the tumor ROIs while points shown as squares represent hemispheric data. The dotted and bold lines indicate relative CBV and CBF, respectively.

A one-sample t-test was performed on the normalized relative CBV and CBF ratios. The mean normalized relative CBV ratios for hemispheric regions and tumor ROI were 1.01 (standard error [SE] $\pm$ 0.02, $P = 0.55$) and 1.45 (SE $\pm$ 0.09, $P < 0.0001$), respectively. The mean normalized relative CBF ratios for hemispheric regions and tumor ROIs were 0.99 (SE $\pm$ 0.02, $P = 0.48$) and 1.34 (SE $\pm$ 0.07, $P < 0.0001$), respectively. The ROIs represent statistically significant increases of relative CBV and CBF by 43.6% and 35.4%, respectively,

Table 3
Data Quality Inspection Table for Patient No. 16

Study	Percent fit failure	Signal min time	Signal full width at half min	Percent susceptibility drop
Baseline	2.55	30.40	13.54	82.14
8 weeks	2.65	36.10	17.70	53.70
16 weeks	3.45	28.50	14.40	52.26
24 weeks	2.40	30.40	16.11	74.92
32 weeks	12.72	76.00	57.85	61.95

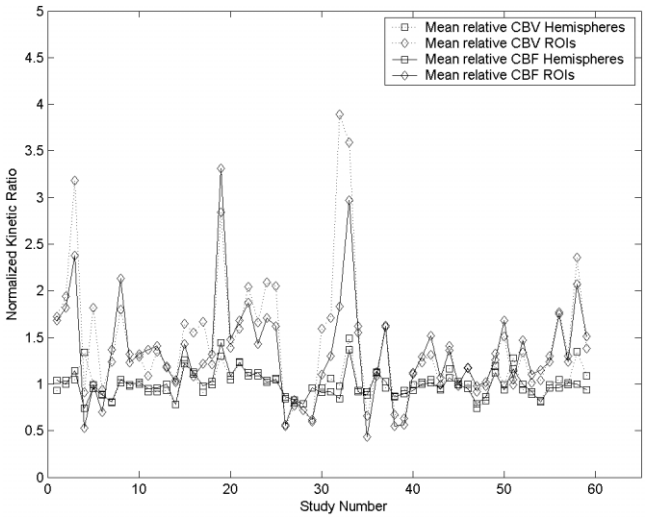

Figure 3. The normalized kinetic parameters for studies in this work showing the relative CBV and relative CBF for hemispheric data and tumor ROIs.

when analyzed by tumor defined ROI in recurrent malignant glioma. These increases clearly suggest that localized measurements of kinetic parameters, i.e., over specific ROIs, are of greater value in assessing localized tumor perfusion parameters than hemispheric measurements.

An individual example is illustrated in Fig. 4. The post-contrast T1-weighted image with enhancement at the location of the tumor is shown in Fig. 4a. The average signal across the 65 time points in the functional acquisition that generates the susceptibility curve is shown in Fig. 4b. The normalized kinetic ratios (tumor ROI to contralateral ROI) for the perfusion CBV maps are determined if the data set met threshold values. The bar chart shows four clusters (one for each study, chronologically arranged) each with normalized ratios from seven contiguous anatomic slices. This patient showed a partial clinical response while on this trial. The parametric CBV and CBF maps generated by the perfusion analysis are shown in Fig. 4d and 4e, respectively.

In this trial the patient response criteria was defined as follows: 1) complete response (CR), complete disappearance of the entire tumor on MRI images, off glucocorticoids, with a stable or improving neurologic examination for at least four weeks; 2) partial response (PR), ≥50% reduction in tumor size in volumetric MRI studies, on a stable or decreasing dose of glucocorticoids, with a stable or improving neurologic exam for at least four weeks; 3) progressive disease (PD), progressive neurologic abnormalities not explained by causes unrelated to tumor progression, or >25% increase in

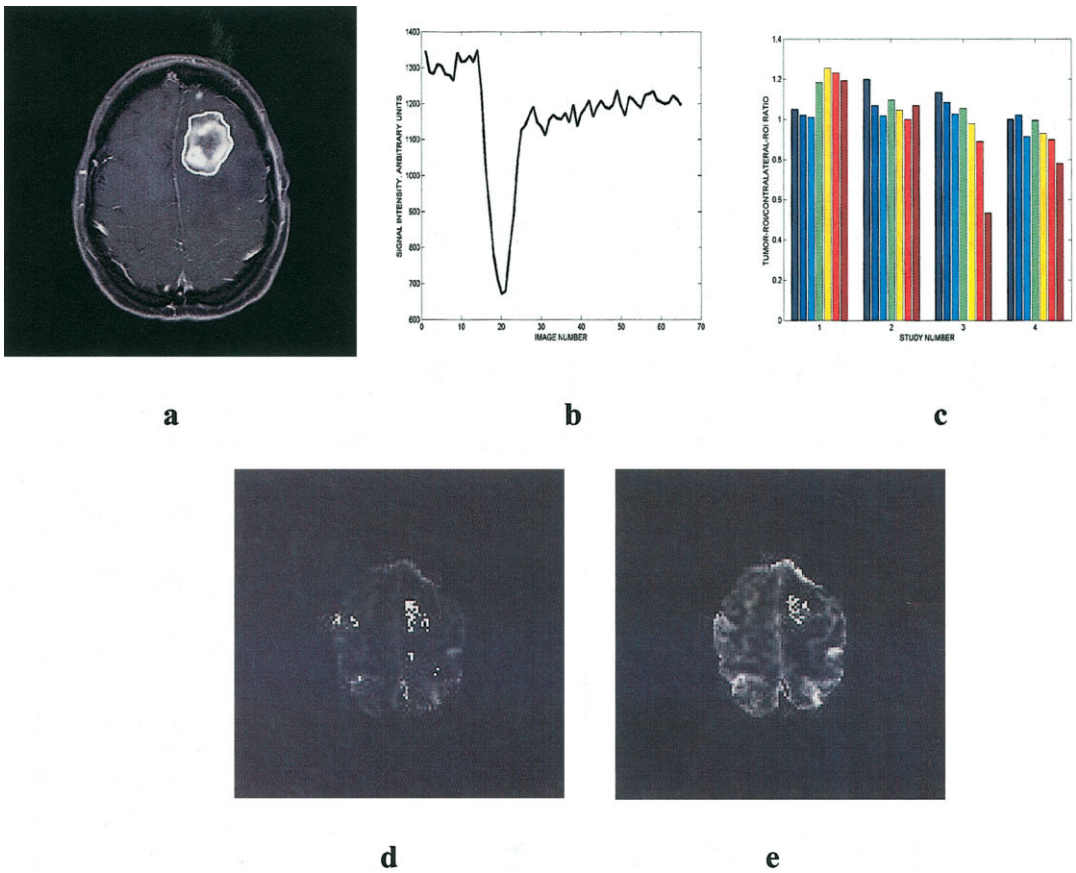

Figure 4. Subject 16, dose level 3. **a:** The T1 post-contrast image indicating the anatomic location of the tumor. **b:** Graph showing the average signal intensity over the functional study. **c:** The normalized CBV values for the seven slices over four studies (studies were eight weeks apart). **d:** The perfusion CBV map. **e:** The perfusion CBF map. This patient showed a partial clinical response to the angiogenesis inhibitor. Note: a, b, d, and e are from the baseline study.

Table 4
Change in CBV and CBF: Comparison of Patients by Clinical Response

Parameter	Clinical response	Mean	Standard error	*P*-value
CBV	Stable	−0.07	0.03	0.08
	Progression	+0.05	0.05	
CBF	Stable	−0.08	0.04	0.009
	Progression	+0.23	0.07	
CBV	All Response	−0.19	0.08	0.08
	Progression	+0.05	0.05	
CBF	All Response	−0.15	0.05	0.004
	Progression	+0.23	0.07	

MR image tumor volume; and 4) stable disease (SD), a patient whose clinical status and MRI volumetrics did not meet the criteria for either PR or PD.

In order to correlate the changes in relative CBV and CBF with the clinical response, the normalized kinetic ratios were tracked longitudinally for patients with multiple perfusion studies. We compared patients with PD with those that had any kind of clinical response (CR) (CR + PR + SD = six cases) as well as PD with stable response. We tested changes in relative CBV and CBF by measuring the maximal and minimal differences with respect to the pre-treatment baseline scan. For patients with only two studies, the maximal and minimal differences would be the same. For statistical analysis, we utilized the minimal difference as it is a more rigorous value and less susceptible to bias. The mean changes from baseline are summarized in Table 4.When comparing stable patients and those with PD, the difference between CBV changes is 0.12 ($P = 0.08$) and difference between CBF changes is 0.31 ($P = 0.009$). When comparing PD patients and those that exhibited any response, the difference between CBV changes is 0.24 ($P = 0.08$) and difference between CBF changes is 0.38 ($P = 0.004$). These suggest that the change in relative CBF is the most significant statistical metric for differentiating between patients' clinical responses.

Radiographic return to normalcy was observed by tracking the CBF and CBV of patients that demonstrated a clinical response to anti-angiogenic therapy. In addition, patients with documented clinical progression demonstrated increases in the relative CBV and/or CBF. We illustrate four such cases that are summarized in Table 5. Patient 4 showed an 8% and 15% decrease in relative CBV and CBF, respectively, from baseline to most recent study. This patient exhibited a stable clinical response. Patient 16 had a 28% drop in CBV and 18% drop in CBF from baseline to most recent study, and clinically showed PR. Patient 36 showed a 35% and 27% decreases in relative CBV and CBF, respectively, and a complete clinical response. Patient 2 had PD by clinical criteria and a 22% increase in relative CBF.

DISCUSSION

Malignant brain tumors are characterized histologically as very heterogeneous tumors with areas of intense tumor proliferation, neovascularization, and regions of tumor necrosis (31). The process of tumor-associated vascular proliferation or angiogenesis is believed to be essential for malignant progression (1–3). The molecular steps important for glioma angiogenesis are being elucidated resulting in therapeutic opportunities directed toward this process. The assessment of anti-angiogenic agents in early phase clinical trials has provided unique challenges. The traditional early phase clinical trial design has relied on the development of toxicity to define doses for further efficacy testing. For anti-angiogenic agents, toxicity may be mild and doses associated with toxicity may not necessarily be those associated with biological activity. As a result, clinical investigation with this class of agents may best be served by determining the optimal biological dose (OBD) as opposed to the maximum tolerated dose. The determination of an OBD would require, as a gold standard, the quantitation of a molecular target or phenotypic change in order to be valid. This validation typi-

Table 5
Kinetic Parameters in Patients With Clinical Response to EMD 121974

Study	Patient # 2 progressive disease		Patient # 4 stable response		Patient # 16 partial response		Patient # 36 complete response	
	CBV	CBF	CBV	CBF	CBV	CBF	CBV	CBF
Baseline	1.33	1.29	1.08	1.19	1.18	1.19	1.37	1.31
+8 weeks	1.24	1.57	1.18	1.21	1.07	1.16	0.88	0.95
+16 weeks	–	–	1.06	1.13	0.85	0.95	–	–
+24 weeks	–	–	1.07	1.29	0.84	0.97	–	–
+32 weeks	–	–	1.14	1.10	–	–	–	–
+40 weeks	–	–	0.99	1.01	–	–	–	–

cally requires obtaining tissue from patients before treatment and at various time points during treatment. This is not possible with primary brain cancer for several reasons including risk, expense, and biopsy bias. The utilization of non-invasive imaging methodologies like DSC-MRI to assess tumor associated angiogenesis is thus essential for the advancement of novel therapies. Their validation will ultimately require correlation with clinical outcomes that will be possible once the phase I trials are complete.

To utilize noninvasive imaging methods in multi-institution clinical trials, we must first define the requirements of quality and methods for the central analysis of such data sets. The extraction of information on perfusion parameters such as CBV and CBF from DSC-MRI studies and the comparison of this information from one patient to another, from one institution to another, or from one point in time to another require a method to determine if a study meets certain criteria. The susceptibility curve from which perfusion data is extracted is subject to not only differences in the tissue microvascular environment but also to technical factors such as contrast injection rate, contrast concentration, and MR sequence parameters as well as patient factors such as cardiovascular parameters. The standardization of technical and acquisition sequence factors is a clear step in quality control. However, this alone is inadequate for the comparison of perfusion values across institutions and even longitudinally within the same patient. Malignant gliomas are extremely heterogeneous both at the histologic and imaging levels. The utility of small ROIs (several pixels) placed in various regions of the tumor will not allow an unbiased evaluation of relative CBV and CBF in the setting of an anti-angiogenic trial. We recommend the use of a ROI, defined on the basis of the complete tumor cross-section, in the post-contrast T1 images.

An issue that merited study was the choice of imaging sequence for our data acquisition. The gradient recalled EPI sequence with interleaved acquisition was chosen for acquiring functional perfusion data. Gradient-echo (GE) EPI and SE-EPI have shown different sensitivities to vessel size, indicating a variation in relative CBVs based on different tumor sizes and grades. GE-EPI and SE-EPI techniques were compared for detecting low- vs. high-grade gliomas and the GE-EPI technique seemed more useful for detecting low- vs. high-grade gliomas than the SE-EPI technique (32). In a similar study comparing echo-planar sequences for perfusion-weighted MRI based on image quality, artifacts, signal-to-noise ratio (SNR), and signal attenuation-to-noise ratio, it was shown that at lower field strengths (2.35 T and less), GRE-EPI sequences are best suited for perfusion studies because they have the highest SNR and T2* sensitivity (33).

Malignant gliomas characteristically have disruption of the blood-brain barrier resulting in the extravasation (34) of gadolinium-based contrast agents into the interstitial space. This may have unwanted effects leading to the underestimation of the CBV and/or CBF. These include both T1 and T2* effects. The T1 effect caused by contrast extravasation is seen as a rise in the signal intensity above baseline after the initial drop (35). This is usually overcome by using techniques like limited integration methods. The T2* effects result in an incomplete recovery of the signal-time intensity curve to baseline and, if excessive, may not allow a gamma variate fit of the data to be achieved. The accuracy of gamma variate fitting in the context of similar contrast-enhanced MRI techniques has been documented earlier (36). Several computational models are available to partially correct for such effects but these are computationally demanding. An alternate solution is to administer a small pre-dose of contrast agent to saturate brain tissue and minimize contrast agent leakage during the actual perfusion study. The use of statistical thresholds to help define MRI parameters like susceptibility characteristics as well as CBV and CBF computations is one option for enhancing the quality of study results. Of the data sets in our study, 82% (59 out of 72) passed the statistical quality and reliability thresholds. We believe that statistically limiting our data based on susceptibility characteristics and gamma-variate fit failure rate will standardize results across the institutions involved in the trial.

DSC-MRI has been shown to be a useful tool in assessing brain tumor angiogenesis. Perfusion analyses of primary brain tumors have demonstrated abnormal parameters associated with higher grade (WHO grade III and IV) tumors when compared to normal or low grade (WHO I) (6,31). In addition, in vivo studies using animal xenograft models of human gliomas support elevated perfusion parameters and are able to correlate these findings with histologic measures of angiogenesis (25). Our data is the first report of T2* perfusion results for an early phase clinical trial and includes the evaluation of 36 patients with over 70 studies. Malignant gliomas have significantly altered perfusion parameters that vary widely throughout the tumor. The normalization of perfusion results was accomplished by using either the tumor hemisphere to the uninvolved hemisphere or a tumor defined ROI to a contralateral uninvolved ROI. Hemispheric information about perfusion that was easier to define and less time consuming did not lead to significant inferences about perfusion properties either at baseline or over time. The 34% to 42% increase in kinetic indices when using a specific ROI indicate that ROI analysis is useful for studying changes in tumor blood volume/flow. The changes in blood flow and volume merit a detailed statistical analysis and possibly correlation with clinical outcomes of the patients. This could yield critical information about tumor proliferation or regression non-invasively. The efficacy of angiogenesis inhibitors like EMD 121974 can be gauged by non-invasively measuring local relative CBV and CBF. In select patients who were on angiogenesis inhibitor treatment, significant reductions in the perfusion parameters in surrounding areas of the primary tumor were observed. Patients were imaged before the start of the administration of EMD 121974 and at subsequent intervals of eight weeks. The temporal changes in normalized kinetic indices are suggestive of a slow decrease in blood vessel proliferation in the tumor. The normalized kinetic ratios were tracked over a period of time over several anatomic slices. The ratios for CBV and CBF decreased significantly. The relative

CBV ratios decreased 8% to 36% and relative CBF ratios decreased 15% to 28% in select patients that demonstrated a clinical response. In contrast, patients with progressive disease clinically demonstrated increases in these values. The variations in relative CBV and CBF across several anatomic slices indicate that these perfusion indices are very heterogeneous over the tumor volume. This is as suggested by earlier reports on tumor histology although we did not attempt to establish such a correlation in this study. Our results do indicate that in the assessment of anti-angiogenic agents in early phase clinical trials, the definition of the ROI is of critical importance.

The use of DSC-MRI is a viable method for the assessment of perfusion parameters in the evaluation of anti-angiogenic agents. This is confirmed for the measure of relative CBF by a statistically significant correlation with clinical responses. This is suggested for relative CBV that approached significance in our study. As we progress with further clinical evaluations with increased sample size, statistical correlations will be more robust. The present study included patients with recurrent malignant glioma, of which nine were initially diagnosed as anaplastic astrocytoma (AA, grade III) and 24 as GBM (grade IV). An analysis of relative CBV and CBF did not demonstrate statistically significant differences between GBM and AA. As the patient population eligible for this trial was at recurrence, the lack of a difference may be reflected in the transformation or progression of many of the AA cases to GBM. CBF and CBV may not distinguish between these two grades of malignant tumors.

Specialized pixel coregistration techniques could help in monitoring the same location of a patient's brain images over a period of time and would potentially present more accurate localized hemodynamics. In the case of patients who showed a clinical response to the angiogenesis inhibitor, functional perfusion MRI studies showed a decrease in normalized ratios of both relative blood volume and flow. The reduction of these ratios shows that these patients had similar hemodynamics in the tumor ROI and in areas of healthy tissue. These ratios typically dropped to 1.00 and below. Ratios below 1.0 are possible effects of radiation necrosis, edema, or surgery. Radiographic response of brain tumor patients using DSC-MRI can thus be used to noninvasively assess patient progress and also the efficacy of the drug provided studies are subjected to rigorous quality evaluations.

In summary, we used DSC-MRI data from a multiinstitution trial to define the requirements for quality and reliability thresholds that permit analyses of perfusion parameters. These parameters are abnormal in malignant gliomas when analyzed with a defined ROI and may be utilized to evaluate the biological activity of anti-angiogenic drugs in efficacy trial testing.

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