Published in final edited form as:

Magn Reson Imaging. 2013 July ; 31(6): 900–910. doi:10.1016/j.mri.2012.12.002.

A Reference Agent Model for DCE MRI can be used to quantify the relative vascular permeability of two MRI contrast agents

Julio Cárdenas-Rodríguez^{1,#}, Christine M. Howison², Terry O. Matsunaga³, and Mark D. Pagel^{1,3,4,5}

¹Department of Chemistry and Biochemistry, University of Arizona, Tucson AZ

²Arizona Research Laboratories, University of Arizona, Tucson AZ

³Department of Medical Imaging, University of Arizona, Tucson AZ

⁴Department of Biomedical Engineering, University of Arizona, Tucson AZ

⁵University of Arizona Cancer Center, Tucson AZ

Abstract

Dynamic Contrast Enhancement (DCE) MRI has been used to measure the kinetic transport constant, K^{trans} , which is used to assess tumor angiogenesis and the effects of anti-angiogenic therapies. Standard DCE MRI methods must measure the pharmacokinetics of a contrast agent in the blood stream, known as the Arterial Input Function (AIF), which is then used as a reference for the pharmacokinetics of the agent in tumor tissue. However, the AIF is difficult to measure in pre-clinical tumor models and in patients. Moreover the AIF is dependent on the Fahraeus effect that causes a highly variable hematocrit (Hct) in tumor microvasculature, leading to erroneous estimates of K^{trans} . To overcome these problems, we have developed the Reference Agent Model (RAM) for DCE MRI analyses, which determines the ratio of K^{trans} values of two contrast agents that are simultaneously co-injected and detected during a single DCE-MRI scan session. The RAM obviates the need to monitor the AIF because one contrast agent effectively serves as an internal reference in the tumor tissue for the other agent, and it also eliminates the systematic errors in the estimated K^{trans} caused by assuming an erroneous Hct . Simulations demonstrated that the RAM can accurately and precisely estimate the relative K^{trans} ($R^{K^{trans}}$) of two agents. To experimentally evaluate the utility of RAM for analyzing DCE MRI results, we optimized a previously reported multiecho ^{19}F MRI method to detect two perfluorocarbon contrast agents that were co-injected during a single in vivo study and selectively detected in the same tumor location. The results demonstrated that RAM determined $R^{K^{trans}}$ with excellent accuracy and precision.

Keywords

Dynamic Contrast Enhanced MRI; Permeability; Reference Agent Model; Pharmacokinetics; ^{19}F MRI; Perfluorocarbons; Linear Models

© 2012 Elsevier Inc. All rights reserved.

Corresponding author: Mark D. Pagel Ph.D., Arizona Cancer Center, Room 4949, 1515 N. Campbell Ave, Tucson, AZ. 85724-5024, Tel: (520)-404-7049, mpagel@u.arizona.edu.

[#]Current Affiliation: Institute of Imaging Science and Department of Radiology, Vanderbilt University, Nashville, TN. USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Angiogenesis is critical for the sustained growth and metastasis of solid tumors, which negatively affects patient outcomes (1,2). Anti-angiogenic therapies are often cytostatic and do not decrease tumor volume, thus evaluations of vascular permeability are often required to assess early therapeutic response (3). Dynamic Contrast Enhanced MRI (DCE MRI) has been used to detect changes in vascular permeability, typically represented by the transfer constant K^{trans} , by monitoring the rate of uptake and/or washout of a MRI contrast agent (CA) in the tumor tissue (4). The results from many DCE MRI studies have been correlated with microvessel densities (5), tumor grades (6), expression of VEGF (7), microarray gene expression analyses (8), tumor oxygenation (9), interstitial fluid pressure measurements (10), and biopsies (11).

Despite these promising results, DCE MRI has inherent limitations for quantitative pre-clinical and clinical cancer studies (12). For example, the concentration of a CA in blood plasma must be monitored to provide an Arterial Input Function (AIF) that is required to determine K^{trans} using traditional DCE MRI analysis methods (13). Identifying a suitable artery or vein within the MR image can be challenging, particularly for DCE MRI studies of mouse models of human cancers. Motion artifacts and in-flow effects can compromise the quality of the AIF. Furthermore, MR images must be acquired at a rate ~ 5 sec per image to accurately capture the AIF for accurate determinations of K^{trans} (14,15).

An often overlooked limitation of DCE MRI is the inability to account for the exact value of the hematocrit in the tumor microvasculature. Most DCE MRI studies assume that the arterial hematocrit is 40%, which is an acceptable estimate for patients with cancer (16). Moreover, most studies assume that the hematocrit is a single value throughout the tumor and normal tissues. However, the hematocrit decreases as the vessel diameter decreases, so that the hematocrit in tumor microvasculature ranges between 20–80% of the hematocrit of a typical artery that is used for blood sampling (17). This physiological condition is known as the Fahraeus effect (18). The measurement of K^{trans} is linearly and inversely dependent on the hematocrit, so that an erroneous overestimation of the microvascular hematocrit directly translates to an erroneous underestimation of K^{trans} (19). Similar effects of an erroneous hematocrit on the underestimation of K^{trans} have been reported, although these reports have been limited to the variability of large-vessel hematocrit between patients and have not addressed variability of capillary hematocrit in a single tumor (20,21). A low hematocrit may contribute to hypoxia that can stimulate angiogenesis, increase vascular permeability and lead to high values of K^{trans} . Therefore, tumor regions with the highest K^{trans} may have the most underestimated K^{trans} values. This underestimation can be as great as 36%, which may partly explain why a 40% change in DCE MRI measurements is typically required to detect a statistically significant change in tumor angiogenesis in carefully controlled pre-clinical DCE MRI studies (22,23).

We propose a new model that addresses these limitations of DCE MRI. This new model, termed the Reference Agent Model (RAM), compares the pharmacokinetics of two CAs in the same tumor tissue, so that one agent may be used as a reference for the second agent. The RAM does not require measurement of the AIF. Because both CAs in the same tumor location must experience the same hematocrit, a ratiometric comparison of DCE MRI of both agents is independent of the hematocrit. More generally, a ratiometric approach has potential to cancel other characteristics of DCE MRI measurements that complicate the interpretation of the results. Therefore, this ratio of K^{trans} , known as $R^{K^{trans}}$, should accurately represent the relative permeabilities of the two CAs. The derivation of RAM is presented in this report, along with computer simulations that evaluated the sensitivity of

RAM to signal-to-noise ratio, temporal resolution, and extreme values of K^{trans} and the extracellular extravascular fractional volume (v_e).

To implement RAM, two MRI contrast agents must be selectively detected during a single DCE MRI scan session in the same tumor region for ROI analyses, or within each pixel within the image of the tumor for pixel-wise analyses. Because two co-injected T_1 and/or T_2 CAs are difficult to selectively detect with MRI, we have developed ^{19}F CAs and optimized ^{19}F MRI methods to perform simultaneous ^{19}F DCE MRI of two CAs in a mouse model of breast cancer. Our strategy is based on the ^{19}F MRI multi-echo approach developed by Girardeau, et al., for the highly sensitive detection of a single ^{19}F nanoemulsion in vivo (24,25). However, instead of detecting a single ^{19}F CA, we investigated the co-injection of two different nanoemulsions and selective detection of each nanoemulsion in an interleaved fashion. The results of this ^{19}F DCE MRI study were analyzed using the RAM.

THEORY

Derivation of the Model

Our derivation of the RAM uses the notation and symbolic conventions described by Tofts et al. (26). The RAM shares similarities with the Reference Region Model for DCE MRI, and therefore our derivation also uses the notation and symbolic conventions described by Yankeelov et al. (27). The differential equations [1] and [2] describe the pharmacokinetic behavior of two contrast agents (CA) within a voxel. In this equation, $Cb_{CA-1}(t)$ and $Ct_{CA-1}(t)$ are the concentrations of one CA (CA-1) at time t in the blood and the tissue of interest respectively; $K^{trans,CA-1}$ is the transfer constant (min^{-1}) between the blood and the Extravascular Extracellular Space (EES) of the tissue of interest (TOI) for CA-1; v_e is the extravascular-extracellular fractional volume of TOI; and Hct is the hematocrit (fraction of blood volume occupied by red blood cells). A differential equation for the second contrast agent (CA-2) is analogous (Eq. [2]). To emphasize the role of the hematocrit in this derivation, we have elected to include $Cb(t)$ and Hct in these differential equations rather than use $Cp(t)$, which is a term that is more commonly used in derivations of DCE MRI theory. Yet $Cp(t)$, $Cb(t)$, and Hct are related through Eq. [3]. For this derivation, we assumed that both agents are detected within the same TOI, thus v_e and Hct are the same for both agents.

$$\frac{dCt_{CA-1}(t)}{dt} = K^{trans,CA-1} \cdot \frac{Cb_{CA-1}(t)}{(1-Hct)} - \frac{K^{trans,CA-1}}{v_e} \cdot Ct_{CA-1}(t) \quad [1]$$

$$\frac{dCt_{CA-2}(t)}{dt} = K^{trans,CA-2} \cdot \frac{Cb_{CA-2}(t)}{(1-Hct)} - \frac{K^{trans,CA-2}}{v_e} \cdot Ct_{CA-2}(t) \quad [2]$$

$$Cp(t) = \frac{Cb(t)}{(1-Hct)} \quad [3]$$

The AIF can be eliminated from Eq. [1] by assuming that both agents show the same AIF, solving Eq. [2] for $Cb(t)/(1-Hct)$ (Eq. [4]), and substituting Eq. [4] into Eq. [1], resulting in Eqs. [5a] and [5b].

$$\frac{Cb(t)}{(1-Hct)} = \frac{1}{K^{trans,CA-2}} \cdot \frac{dCt_{CA-2}(t)}{dt} + \frac{1}{v_e} \cdot Ct_{CA-2}(t) \quad [4]$$

$$\frac{dCt_{CA-1}(t)}{dt} = \frac{K^{trans,CA-1}}{K^{trans,CA-2}} \cdot \frac{dCt_{CA-2}(t)}{dt} + \frac{K^{trans,CA-1}}{v_e} \cdot Ct_{CA-2}(t) - \frac{K^{trans,CA-1}}{v_e} \cdot Ct_{CA-1}(t) \quad [5a]$$

$$\frac{dCt_{CA-1}(t)}{dt} = \frac{K^{trans,CA-1}}{K^{trans,CA-2}} \cdot \frac{dCt_{CA-2}(t)}{dt} + \frac{K^{trans,CA-1}}{v_e} \cdot [Ct_{CA-2}(t) - Ct_{CA-1}(t)] \quad [5b]$$

Integrating both sides of Eq. [5b] and assuming that the initial concentrations of CA-1 and CA-2 are equal to zero yields the working equation for the RAM (Eq. [6]) where $R^{K^{trans}}$ is the relative $K^{trans,CA-1}$ compared to $K^{trans,CA-2}$, and $k_{ep,CA-1}$ is the rate constant (min^{-1}) between the EES of the TOI and plasma for CA-1.

$$Ct_{CA-1}(T) = \frac{K^{trans,CA-1}}{K^{trans,CA-2}} \cdot Ct_{CA-2}(T) + \frac{K^{trans,CA-1}}{v_e} \cdot \left[\int_0^T Ct_{CA-2}(t) dt - \int_0^T Ct_{CA-1}(t) dt \right] \quad [6a]$$

$$Ct_{CA-1}(T) = R^{K^{trans}} \cdot Ct_{CA-2}(T) + k_{ep,CA-1} \cdot \left[\int_0^T Ct_{CA-2}(t) dt - \int_0^T Ct_{CA-1}(t) dt \right] \quad [6b]$$

The RAM can be expressed in matrix form (Eqs. [7] and [8]). The elements of column two of vector the M in Eq. [8] can be approximated by numerical integration. Eq. [8] is a system of linear equations, which can be solved for the elements of vector b using linear algebra.

$$\vec{A} = M^r \cdot b^r \quad [7]$$

where

$$\vec{A} = \begin{pmatrix} Ct_{CA-1}(T1) \\ Ct_{CA-1}(T2) \\ \mathbf{M} \\ Ct_{CA-1}(Tn) \end{pmatrix}; b^r = \begin{pmatrix} R^{K^{trans}} \\ k_{ep,TOI} \end{pmatrix}; M^r = \begin{pmatrix} C_{CA-2}(T1) & -\left[\int_0^{T1} Ct_{CA-2}(t) dt \right] & -\left[\int_0^{T1} Ct_{CA-1}(t) dt \right] \\ \mathbf{M} & \mathbf{M} & \mathbf{M} \\ C_{CA-2}(Tn) & -\left[\int_0^{Tn} Ct_{CA-2}(t) dt \right] & -\left[\int_0^{Tn} Ct_{CA-1}(t) dt \right] \end{pmatrix} \quad [8]$$

Interpretation of $R^{K^{trans}}$ —Equation [10] shows the most general definition of K^{trans} , where P is the total permeability of the capillary wall (cm min^{-1}); S is the surface area per unit mass of tissue ($\text{cm}^2 \text{g}^{-1}$); F is the flow rate of whole blood per unit mass of tissue ($\text{mL g}^{-1} \text{min}^{-1}$), ρ is the density of tissue (g mL^{-1}), and E is the extraction ratio, defined in Eq. [11] (6). This definition of K^{trans} is reduced to simpler cases under very specific conditions. If the flux of the CA across the tumor endothelium has low permeability relative to flow, then K^{trans} can be approximated by Eq. [13], known as the permeability-limited model of DCE MRI.

$$K^{trans} = E \cdot F \cdot \rho \cdot (1 - Hct) \quad [10]$$

$$E = 1 - e^{-PS/F(1-Hct)} \quad [11]$$

$$E; PS/F \quad \text{when } P^*S \ll F \quad [12]$$

$$K^{trans} = P \cdot S \cdot \rho \quad \text{when } P^*S \ll F \quad [13]$$

The CAs used in this study fulfill the conditions for the permeability-limited model (4). If two large CAs are simultaneously detected in the same TOI, their ratio of K^{trans} values can be expressed as $R^{K^{trans}}$ (Eq. [14]), where P_{CA-1} and P_{CA-2} are the apparent permeabilities of the TOI for CA-1 and CA-2, respectively. Because both agents experience the same S and ρ , $R^{K^{trans}}$ is simply the relative permeability of the two contrast agents.

$$R^{K^{trans}} = \frac{K^{trans,CA-1}}{K^{trans,CA-2}} = \frac{P_{CA-1} \cdot S \cdot \rho}{P_{CA-2} \cdot S \cdot \rho} = \frac{P_{CA-1}}{P_{CA-2}} \quad [14]$$

METHODS

Simulations

Computer simulations were used to study the effect of temporal resolution, a wide range pharmacokinetic constants, and statistical noise on the accuracy and precision of RAM to estimate $R^{K^{trans}}$ and $k_{ep,CA-1}$. First, the arterial input function for the ^{19}F nanoemulsions used in this study was experimentally measured (for details, see the section below titled “Determination of the AIF using in vivo ^{19}F DCE MRI”) and a biexponential equation was fit to these results (Eq. [15]).

$$C_p(t) = A \cdot e^{(-\alpha \cdot t)} + B \cdot e^{(-\beta \cdot t)} \quad [15]$$

Although this experimental measurement produced relative values of the distribution volumes of the AIF (A and B of Eq. [15]), converting these relative values to absolute volumes requires careful calibration of MR signals relative to known volumes, which is technically challenging. Therefore, we used previously reported values of A and B with our experimentally determined values of α and β to simulate the AIF from 0 to 30 min (28,29). The final values for each parameter were $A=24.0$ kg/L; $\alpha=16.54$ min $^{-1}$; $B=5.5$ kg/L; $\beta=0.03935$ min $^{-1}$. Next, the temporal dependence of contrast agent concentrations, $C_{tCA-1}(t)$ and $C_{tCA-2}(t)$, were calculated using the standard Tofts model and the simulated AIF with K^{trans} equal to 8.2×10^{-3} min $^{-1}$ and 4.3×10^{-3} min $^{-1}$ for CA-1 and CA-2, respectively (Eq. [16]) (26). These values assigned to K^{trans} are characteristic for ^{19}F nanoemulsions with diameters of 225 and 293 nm in animal models of solid tumors (30,31). The v_e was set to 0.5 mL/mL for both agents, which has been reported previously for human tumors and animal models of breast cancer (32).

$$Ct(T) = K^{trans} \int_0^T C_p(t) \cdot e^{\left[-\frac{K^{trans}}{v_e}(T-t)\right]} dt \quad [16]$$

Two sources of systematic error were explored using the simulated data with no noise added. To test the effect of temporal resolution (Δt), the AIF, $Ct_{CA-1}(t)$ and $Ct_{CA-2}(t)$ were down-sampled from their initial Δt of one second to $\Delta t=0.5, 2.0$ and 4.0 minutes. The parameters R_{Ktrans} and $k_{ep,CA-1}$ were then estimated using the simulated data at each new Δt . To test the effect of extreme pharmacokinetic constants, R_{Ktrans} and $k_{ep,CA-1}$ were estimated from simulated data generated with $K^{trans,CA-1}$ ranging from 0.001 to 0.150 min^{-1} in steps of 0.003 min^{-1} while fixing $K^{trans,CA-2}$ at $4.3 \times 10^{-3} \text{ min}^{-1}$ and fixing v_e at 0.5 mL/mL . In a similar analysis, v_e was changed from 0.10 to 0.9 mL/mL in steps of 0.005 mL/mL while $K^{trans,CA-1}$ and $K^{trans,CA-2}$ were fixed at 8.2×10^{-3} and $4.3 \times 10^{-3} \text{ min}^{-1}$, respectively.

The combined effect of signal-to-noise ratio (SNR) and Δt on the accuracy and precision of RAM was studied by adding Gaussian noise to the $Ct_{CA-1}(t)$ and $Ct_{CA-2}(t)$ curves at $\Delta t=0.5, 2.0$ and 4.0 min to produce SNRs from 5 to 100 . The SNR was defined as $SNR=S_{\max}/\sigma$, where $S_{\max}$ represents the maximum signal of each curve and σ is the standard deviation of the noise (SD). This process was repeated 1000 times for each condition of SNR and Δt . The accuracy of parameter estimation was evaluated in terms of the mean percentage error (Eq. [17]), and the precision was evaluated using the standard deviation of the percentage error (Eq. [18]), where $\hat{P}_{ij}$ is the estimated value of the j^{th} data of the i^{th} parameter, P_i is the true value of the i^{th} parameter, and N is the number of data points (1000 for this study).

$$\mu_{error}=100 \cdot \frac{1}{N} \left(\sum_{j=1}^N [(\hat{P}_{ij}-P_i)/P_i] \right) \quad [17]$$

$$\sigma_{error}=100 \cdot \sqrt{\frac{1}{N} \sum_{j=1}^N (\hat{P}_{ij}-\mu_{error})^2} \quad [18]$$

Experimental

Preparation of ^{19}F nanoemulsions—A total of 14 mg of dipalmitoylphosphatidylcholine (DPPC), 5 mg of $1,2$ -dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] ($16:0$ -PEG2000-PE) and 1 mg of $1,2$ -dipalmitoylphosphatidic acid (DPPA) were dissolved in 1 mL of chloroform. The organic solvent was evaporated in a rotary evaporator at 35°C to obtain a thin film, and solvent traces were removed by maintaining the lipid film under a vacuum overnight. The film was hydrated with 1.0 mL of PBS buffer using a sonicator (Branson 510, CT, USA) at a 250 W intensity at room temperature for 10 min . This mixture was subjected to 10 freeze-thaw cycles using a liquid nitrogen bath at -196°C and a water bath at 60°C . Next, perfluorinated-15-crown-ether (PCE) or perfluorooctane (PFO) was added at $40\% \text{ v/v}$ to the system, followed by 20 manual extrusions at 60°C through a 100 nm filter (Avanti Lipids, Alabama, USA). Samples of the resulting emulsions were diluted to $1\% \text{ v/v}$ in HEPES buffer to measure their particle sizes using dynamic light scattering (Zetasizer Nano, Malvern, Worcestershire, UK).

Correlation of ^{19}F MR signal and ^{19}F concentration—Phantoms of PCE nanoemulsions that ranged in 0.40 to 25.0 M ^{19}F concentration were prepared by serial dilution. Phantoms of PFO nanoemulsions with the same ^{19}F concentration were also prepared. All MR experiments were performed using a 7T Biospec scanner with a 30 cm bore from Bruker Biospin, Inc. (Ettlingen, Germany) equipped with a double-tuned $^1\text{H}/^{19}\text{F}$ linear birdcage coil with a 3.2 cm diameter from M2M Imaging, Inc. (Cleveland, OH). The

temperature was maintained at 37°C for all MRI studies using an automated system (SA Instruments, Inc.). A series of spin echo MR images of these phantoms were acquired using the following parameters: TR=4000 msec; TE=8.0 msec; NEX=2, excitation pulse=90°; FOV=3.5 cm²; matrix=64×64; slice thickness=4.0 mm; number of slices=1. The MR signal for each concentration of fluorine was fitted to the linear model (Eq. [19]) where Sig is the MRI signal in arbitrary units, [¹⁹F] is the ¹⁹F concentration in Molar, m is the slope and b is the y-intercept.

$$\text{Sig} = m[^{19}\text{F}] + b \quad [19]$$

Measurement of T₁ relaxation time—The T₁ relaxation times of PCE and PFO were measured by acquiring a series of spin-echo MR images with variable TR using the following parameters: TR=150, 300, 600, 900, 1500, 2500, 4000, and 8000 msec; TE=8.01 msec; NEX=4; RARE factor=2; slice thickness=1.0 mm; matrix=64×64; and FOV=3.0 cm². The average signal for a region of interest of the sample was fitted to Eq. [20], where α is the flip angle and A is a constant that includes the proton density and scanner gain. We assumed that TE ≪ T₂^{*}.

$$S = A \left[\frac{\sin \alpha \cdot (1 - e^{-TR/T_1})}{1 - e^{-TR/T_1} \cdot \cos \alpha} \right] \quad [20a]$$

$$S = A (1 - e^{-TR/T_1})_{\text{where } \alpha=90^\circ} \quad [20b]$$

Evaluation of effects of J-modulation and T₂ relaxation time—Inspired by the previous reports by Giraudeau, et al., we investigated the dependence of the T₂ relaxation of PCE and PFO on TE following selective excitation (24,25). A multi spin-echo MRI protocol was used with the following parameters for both agents: TR= 5000 msec; TE/number of echos =15/60, 20/60, 25/60, 30/50, 40/37, 50/30, 60/25, 80/18 msec; NEX=4; excitation pulse=90°; FOV=3.0; matrix=64×64; slice thickness=1.0 mm; number of slices=1. To preserve good SNR, only a maximum of 60 echoes and a maximum effective echo time (TE multiplied by the number of echos) of 1500 msec were used. The r² goodness of fit coefficient was 0.95 for all fittings of the average signal to an exponential function to determine T₂ relaxation times (Eq. [21]). This multi-echo approach has proven to be very sensitive for in vivo detection of a single ¹⁹F nanoemulsion, but has not been used after selective excitation nor for the detection of more than one contrast agent.

$$S = S_0 e^{-TE/T_2} \quad [21]$$

Selective detection of two ¹⁹F nanoemulsions—Spin-echo MR images were obtained using phantoms consisting of 100% PCE, 100% PFO, and a 50:50 mixture of PFO and PCE (v/v). These images were acquired with the same geometry and parameters used to measure T₂ relaxation times, with TE=15 msec. The bandwidth of the 90° excitation pulse was varied between 1,000 and 10,000 Hz. The frequency of the excitation pulse was set to the frequency of PCE, and the bandwidth that maximized the SNR of PCE while limiting SNR 5.0 for PFO was considered to be selective for PCE. The same procedure was used to

determine the bandwidth that was selective for PFO. The bandwidth of the 180° refocusing pulse remained constant at 1000 Hz during these studies.

Animal model and general procedure for in vivo MRI studies—Five female SCID mice weighting approximately 21 g were housed and maintained under specific pathogen-free conditions in accordance with the guidelines of the American Association for Laboratory Animal Care. All experiments met the current regulations and standards of the U.S. Department of Agriculture, the U.S. Department of Health and Human Services, the National Institutes of Health, and the University of Arizona Animal Care Facility. All mice were injected subcutaneously in the right flank with 10^6 MDA-MB-231 cells in 0.1 mL of saline. The tumor was measured every 2 to 3 days using electronic calipers and allowed to grow for 22–26 days to an average volume of 250 mm³ before initiating ¹⁹F MRI studies. Mice were scanned 22–26 days after tumor implantation.

Before the MRI scan, a mouse were anesthetized with 1.5–2% isoflurane in O₂ carrier gas, a 27 G catheter was inserted in the tail vein and physiological monitoring leads were connected to monitor respiration rate and core body temperature during the MRI session. Each mouse was kept at $37.0 \pm 0.2^\circ\text{C}$ during the MRI studies using warmed air that is controlled by an automated temperature-feedback system (SA Instruments, New York, USA). Anatomical positioning was performed prior to injecting the emulsions by acquiring T₁-weighted ¹H images with the following parameters: TR=250 msec; TE=5.0; NEX=1; excitation pulse=90°; FOV= 35 mm²; matrix=128×128; slice thickness=1 mm; number of contiguous slices=11. Each image set was acquired in 32 seconds.

Determination of the AIF using in vivo ¹⁹F DCE MRI—Four mice with a subcutaneous MDA-MB-231 tumor were used to obtain an AIF of a PCE nanoemulsion. A vial with 100 mL of a 40% v/v nanoemulsion of PCE was placed next to the mouse as fiducial marker. A series of dynamic ¹⁹F MR images were acquired using a Spoiled Gradient-echo MRI protocol with the following parameters: TR=150 msec; TE=2.5 msec; NEX=8; excitation pulse=33.3°; FOV= 35 mm²; matrix= 64×64; slice thickness= 4.0 mm; number of contiguous slices=3. The location of the three ¹⁹F image slices was matched to the location of the eleven ¹H image slices that were used for anatomical referencing. Each image set was acquired in 1.28 min, and a series of 25 image sets were acquired for a total acquisition time of 32 min. After the first image set was acquired, 150 µL of a 40% v/v nanoemulsion of PCE were injected manually through a tail vein catheter over 20 seconds. The increase in ¹⁹F MR signal was monitored in a 2×2 voxel region that represented a tail vein. Results from the four mice were averaged, and the average signal-intensity curve was fitted with a bi-exponential model (Eq. [16]) using Matlab (Mathworks Inc., Natick MA).

Selective Detection of two agents during in vivo ¹⁹F DCE MRI—One mouse with a subcutaneous MDA-MB-231 tumor was used to test the selective in vivo detection of PCE and PFO. Two vials each containing 100 µL of a 40% v/v nanoemulsion of PCE and PFO were placed next to the mouse to serve as internal controls. The selective imaging of each CA was achieved by setting the transmitter to the MR frequency of PCE or PFO, while using a pulse bandwidth of 2700 Hz for both excitation and refocusing. This imaging scheme was repeated 18 times using a multi-echo MRI protocol and the following parameters: TR=3000 msec; TE= 25 msec; number of echoes=20; NEX=2; excitation pulse=90°, refocusing pulse=180°, FOV=35 mm²; matrix=64×64, slice thickness=4.0 mm; number of contiguous slices=3. The location of the three ¹⁹F image slices was matched to the location of the eleven ¹H image slices obtained for anatomical referencing. Each image set was acquired in 3.2 min, and the selective excitation of PCE and PFO was iterated, so that the effective temporal resolution for each nanoemulsion was 6.4 min. A total acquisition time of 57.6 minutes was required to obtain 18 images. Mid-way through the acquisition of

the second time point (i.e., the first selective excitation of PFO), 200 μL of a mixture of the emulsions consisting of 3.5 mM of PCE and 4.6 mM of PFO were manually co-injected through a tail vein catheter during 45 seconds. After the data acquisition was finished, all of the echoes were summed for each slice at each time point. Summing the echoes formed a ^{19}F DCE MRI dataset with an increasing SNR as previously reported (24,25). All data were processed using customized Matlab functions. The echoes for each slice at each time point were summed, then an ROI for analysis was determined, and a bioexponential function (Eq. [15]) was fit to the signal as function of time to obtain a smoothed curve for each nanoemulsion. Finally the RAM (Eq. [9]) was fit to the smoothed curves to estimate R^{Ktrans} .

RESULTS

Simulations

The concentration-time curves of two nanoemulsions in tumor tissue were simulated to assess the systematic and random errors when estimating R^{Ktrans} using RAM (Fig. 1). These simulations were constructed using K^{trans} values that have been previously reported for other nanoemulsions in similar tumor models (30,31). Unfortunately, these previous studies did not report the characteristics of the AIF of these nanoemulsions. Therefore, we experimentally measured the exponential decay rates of the biexponential AIF of PCE in four mice (α and β of Eq. [15]). Experimentally measuring the distribution volumes of the AIF (A and B of Eq. [15]), requires careful calibration of MR signals relative to known volumes, which is technically challenging. Therefore, we relied on previously reported values of A and B, and our experimentally determined values of α and β , to construct the AIF for this simulation (30,31).

The accuracy of estimating R^{Ktrans} from the RAM was determined by measuring the systematic error between R^{Ktrans} used to construct the simulated data and the value calculated by the RAM. This systematic error was dependent on the temporal resolution of the DCE MRI acquisition protocol (Fig. 2A). The systematic error was greatest for slow temporal resolutions when R^{Ktrans} was large, which suggests that the rapid pharmacokinetics of one agent cannot be adequately compared with the slower pharmacokinetics of the other agent when temporal resolution is slow. Yet when the pharmacokinetics of both agents was comparable, creating a small to moderate value of R^{Ktrans} , then a slow temporal resolution accurately estimated R^{Ktrans} with a systematic error of less than 1%. The systematic error in estimating R^{Ktrans} also depended on v_e , but this error was less than 1% for all values of v_e for the range of v_e that was evaluated (Fig. 2B).

For comparison, a slow temporal resolution caused a greater systematic error in $k_{ep,CA-I}$ for all values of R^{Ktrans} (Fig. 2C). A low value of v_e also caused a greater systematic error in $k_{ep,CA-I}$ (Fig. 2D). Furthermore, the systematic error in estimating $k_{ep,CA-I}$ was $> 1\%$ for all cases except the fastest temporal resolution, the lowest values of R^{Ktrans} , and the highest values of v_e . These results demonstrated that the effects of a slow temporal resolution can be canceled or reduced by estimating the ratiometric parameter R^{Ktrans} relative to determining the absolute parameter $k_{ep,CA-I}$.

Analyses of the accuracy and precision of R^{Ktrans} measurements were performed by adding Gaussian noise to the simulated concentration-time curves, then using RAM to estimate R^{Ktrans} , and then repeating this process for 1000 iterations. The systematic error represented the level of accuracy, and the standard deviation of these 1000 random errors represented the level of precision (Fig. 3A). The accuracy was within 2% with high SNR greater than 30:1, and within 10% with moderate SNR greater than 15:1 for all temporal resolutions. The precision was within 3.6% with high SNR greater than 30:1 at all temporal resolutions that were tested, and within 3.6% with moderate SNR greater than 15:1 for 0.5 and 2 minute

temporal resolutions. Therefore, the RAM can accurately and precisely determine R^{Ktrans} under conditions of moderate or high SNR. For comparison, the accuracy and precision of estimating $k_{ep,CA-I}$ were highly dependent on SNR (Fig. 3B). These errors in $k_{ep,CA-I}$ were an order of magnitude greater than the errors in R^{Ktrans} , as shown by comparing the scales of the y-axes of Figures 3A and 3B. These results demonstrated that the ratiometric parameter R^{Ktrans} has advantages relative to the absolute parameter $k_{ep,CA-I}$.

Experimental Results

The PCE and PFO nanoemulsions were synthesized using commercially available reagents and standard procedures. As expected, PCE showed a single MR frequency from 20 magnetically equivalent ^{19}F nuclei (Fig. 4A). PFO generated a MR frequency that was 7.58 ppm downfield from the frequency of PCE (2275 Hz at 300 MHz magnetic field strength), which was assigned to the two magnetically equivalent fluoromethyl groups. Due to differences in weight density and fluorine density of the perfluorocarbons, the integral of the PCE signal was 2.22 times higher compared to the fluoromethyl signal of PFO. PFO also showed three MR frequencies at -30.11, -32.3, and -39.5 ppm upfield from the frequency of PCE (9033, 9600, and 10112 Hz), which were assigned to the fluoromethylene groups. The large chemical shift of these fluoromethylene groups provided the opportunity to selectively detect the MR frequency of PCE and the fluoromethyl group of PFO using excitation bandwidths that were much smaller than 9033 Hz, so that the signals from the fluoromethylene groups could be neglected. Therefore, we refer to the fluoromethyl group of PFO in the emulsion as “PFO” for the remainder of this report.

The ^{19}F MRI signal of PCE was found to be linearly correlated with the ^{19}F concentration of the PCE nanoemulsion within the tested range of 0.4 to 12.5 M (Fig. 4B). The average standard deviation of ^{19}F signals from pixels of the image of each phantom was equivalent to 0.10 M based on the signal-concentration calibration. This standard deviation had a range of 0.05 to 0.20 M for all samples, which indicated that this standard deviation was independent of concentration and therefore represented the scanner noise that was independent of the sample. Therefore, a minimum of 0.60 M of ^{19}F nuclei were required to generate a signal with SNR greater than 1 using our MRI scanner and $^1\text{H}/^{19}\text{F}$ transceiver coil. This minimum detection limit equated to a concentration of 0.030 M for the PCE emulsion and .033 M for the PFO emulsion, based on the number of detected ^{19}F nuclei (20 for PCE and 6 for PFO) and average concentration per nanoemulsion particle (61 M for PCE and 71 M for PFO). Although these results established our minimum detection limits, the detection limit for biomedical studies is higher due to biological noise (e.g., motion artifacts).

The estimated T_1 relaxation rate for PCE was 1.031 sec. (with a 95% confidence interval, 95CI, between 0.98 sec. and 1.08 sec.), and the T_1 time of PFO was estimated to be 1.15 sec. (95CI between 1.09 sec. and 1.21 sec.). These relaxation times are significantly different at the 95% confidence level, but this difference leads only to a 3% difference in signal under the conditions of our in vivo studies if both agents exhibit the same spin density.

The selective detection of PCE and PFO nanoemulsions was conducted by optimizing the bandwidth of the 90° excitation pulse while acquiring spin-echo MR images of phantoms (Fig. 4C). A bandwidth of 2700 Hz was found to produce the best SNR for one agent while limiting the SNR of the other agent to less than 5. This design criterion of reducing SNR below 5 for the other agent matched the CNR criterion for statistical significance during our biological studies of the AIF as discussed below. Therefore, a 2700 Hz bandwidth was used for selective detection of each agent during subsequent studies. The bandwidth of the 180° refocusing pulse was found to be inconsequential for selective detection, provided that the

excitation pulse was optimized for selective detection. Yet the bandwidth used for the refocusing pulse in subsequent studies was also set to 2700 Hz for consistency.

To optimize detection sensitivity, we also investigated the T_2 relaxation times of PCE and PFO (Fig. 5). PCE exhibited a T_2 relaxation time of 650 msec in a solution of HEPES buffer. For PFO, the measurement of T_2 relaxation time was discovered to be dependent on the echo time (TE) of the spin-echo imaging protocol. This effect was attributed to J-coupling between the methyl and methylene groups of PFO (24,25). Because the refocusing pulse of the spin-echo protocol was selective for the methyl group, the spin states of the methylene groups were not inverted and therefore the J-coupling was not refocused (data not shown). Longer TE times caused greater J-coupling evolution, leading to greater signal loss. To mitigate this problem, a TE time of 25 msec was used for subsequent studies, which provided equal T_2 relaxation times for both PFO and PCE. The ability to 'tune' the effective T_2 relaxation time of PFO to match PCE removed the complication of different relaxation times from subsequent analyses. Subsequent studies with a TE time of 25 msec acquired and summed 20 spin-echo images that had excellent signal-to-noise. More specifically, the twentieth image had approximately 50% of the signal-to-noise relative to the first image, based on a T_2 relaxation time of 650 msec. This multiecho sequence was also used to measure the in vivo T_2 relaxation time of PCE and PFO, which were 645 msec (95CI between 580 and 715 msec) and 648 msec (95CI between 596 and 719 msec), respectively. The overlapping confidence intervals indicated that the estimated values are not significantly different at the 95% confidence level.

To support the simulations, the AIF of PCE was measured in four mice (Fig. 6). A 2×2 voxel region was identified in the tail that showed strong ^{19}F MRI signal, which was assumed to represent a tail vein (Fig. 6C). As expected, the ^{19}F MRI signal quickly peaked soon after injection, followed by a rapid decrease during the first few minutes and a slow decrease throughout the remainder of the 32-minute scan session (Fig. 6A). The ^{19}F MRI signal was averaged at each time point for the four mice and a biexponential function was fit to the results, resulting in values of $A = 6.11 \times 10^{18}$ (95CI between -3.19×10^{27} and 3.19×10^{27}) and $\alpha = -16.54$ (95CI between -2.72×10^8 and 2.72×10^8) for the first exponential term, and $B = 9.96 \times 10^4$ (95CI between 8.43×10^4 and 1.15×10^5) and $\beta = -0.039$ (95CI between -0.049 and -0.029) for the second exponential term. Although the first exponential term was fit poorly due to poor temporal resolution relative to rapid signal changes, the inclusion of the first exponential term was warranted to produce a good fit to the second exponential term. This analysis was also conducted with the temporal change in ^{19}F MRI SNR (Fig. 6B), which was almost identical to the temporal dependence of ^{19}F MRI signal because the noise level was 1.0 relative to our scale for MRI signals.

Three mice with a subcutaneous MDA-MB-231 tumor were used to perform in vivo ^{19}F DCE MRI and analysis with RAM. A total of 18 multi-echo images were acquired by iterating the selective detection of PCE and PFO, which produced 9 images of each nanoemulsion that were unshuffled to show the temporal dependence of the ^{19}F MRI signals from each nanoemulsion (Fig. 7). The mixture of the nanoemulsions was injected during the acquisition of the second image, so that the first two images showed no significant ^{19}F MR signal. Subsequent images showed a consistently increasing ^{19}F MRI signal in a region of the tumor (Fig. 7A, 7B). These ^{19}F MRI signals in the tumor were converted to ^{19}F concentrations (Fig. 4B). The RAM was used to estimate a value of R^{Ktrans} of 1.16 min^{-1} (95CI between 1.01 and 1.27), which is equal to K^{trans} of PCE divided by K^{trans} of PFO (Fig. 7C). A value of R^{Ktrans} of ~ 1 was expected because the relative diameters of the PCE and PFO nanoemulsions are the same. The root mean square error of the RAM fitting was $2.7 \times 10^{-2} \text{ mM}$, which was a $\sim 1\%$ error relative to the magnitudes of the ^{19}F concentrations that were measured. This small error provided assurance that R^{Ktrans} was

determined with outstanding precision using RAM. In addition, $k_{ep,CA-I}$ was estimated to be $9.1 \times 10^{-3} \text{ min}^{-1}$ (95CI between $8.6 \times 10^{-3} \text{ min}^{-1}$ and $10.1 \times 10^{-3} \text{ min}^{-1}$), which was in the range of expected k_{ep} values for nanoemulsions. Therefore, the in vivo ^{19}F DCE MRI results demonstrated that R^{Ktrans} can be measured with RAM.

For comparison, the MR images of the phantoms of PCE and PFO that were placed next to the mouse showed no changes in SNR during the ^{19}F DCE MRI scan session (data not shown). This validated that ^{19}F signal changes in the tumor were attributed to dynamic changes in the tumor concentration of the nanoemulsions, and were not attributed to potential changes in experimental conditions. Notably, ^{19}F MRI signal was also detected in the center of the abdominal region of the mouse, which indicated that the nanoemulsions accumulated in other areas of the body. The ^{19}F MRI signal was not detected in the bladder, which indicated that the large diameters of the nanoemulsions prevented excretion through the kidneys within the time frame of our DCE MRI studies. This result was expected, as ^{19}F nanoemulsions are typically cleared from the body through the liver or lungs.

DISCUSSION

The analysis of systematic and random errors using simulated results showed that RAM can estimate accurate and precise results for R^{Ktrans} . Yet RAM was shown to be unable to produce the same level of level of accuracy and precision when estimating $k_{ep,CA-I}$. This indicates that RAM is well-suited for determining the ratiometric parameter R^{Ktrans} but is not suited for determining an absolute parameter such as $k_{ep,CA-I}$. Furthermore, RAM was relatively insensitive to the effects of temporal resolution and SNR, which may provide technical advantages during pre-clinical and clinical DCE MRI acquisition protocols.

The average standard deviation of ^{19}F SNR for each time point was 5.2 (Fig. 6B), which was greater than the average standard deviation of SNR measured with phantoms (Fig. 4). This indicated that biological noise contributed to the variability in ^{19}F MRI signal. Yet a $\text{SNR} > 5$ observed during and after the initial signal peak was equivalent to a contrast-to-noise ratio (CNR) > 3.2 (CNR can be considered to be the difference in experimental SNR relative to a baseline SNR of 1.0). A CNR greater than 3.2 has a $>99\%$ probability of being real, which demonstrates that the ^{19}F signals used to generate the AIF for PCE can be assigned to the dynamic changes of the PCE nanoemulsion in the tail vein, even in the presence of the biological noise (33).

The non-linear Reference Region Model (RRM) has been proposed as an alternative method for analyzing DCE MRI results (27,34). The RRM measures the presence of a single MRI contrast agent in two tissues (typically tumor and muscle), and therefore has similarities to the RAM that measures the presence of two MRI contrast agent in a single tissue. Both RAM and RRM have the advantage of canceling effects that may complicate DCE MRI analyses by using a ratiometric approach. For example, both RAM and RRM avoid the need for an AIF. RAM avoids the effect of the hematocrit by comparing results from two MRI contrast agents that inherently experience the same hematocrit. For comparison, the RRM cannot address the problem of the hematocrit, because the hematocrit in tumor and muscle tissues may be unequal, may have different inequalities for different tumor types, and may have different inequalities throughout the notoriously heterogeneous composition of a single tumor. Therefore, RAM has advantages relative to RRM for DCE MRI analyses.

R^{Ktrans} can be most easily applied to permeability-limited conditions of DCE MRI studies, which typically occur with MRI contrast agents that have diameters greater than $\sim 3 \text{ nm}$ (4). Under these conditions, R^{Ktrans} reduces to a simple ratio of vascular permeability for each contrast agent (Eq. [14]). Importantly, the effects of vessel surface area and tissue density

are canceled using this ratiometric approach under permeability-limited conditions, which further improves the physiological interpretation of the results. The traditional DCE MRI evaluations of K^{trans} , and the improved evaluations with RRM, cannot cancel the effects of vessel surface area and tissue density from the analyses.

This study used two ^{19}F nanoemulsions that were intentionally designed to have the same average diameter, so that $R^{K^{trans}}$ is expected to have a value of ~ 1 . The experimentally measured value of $R^{K^{trans}}$ was $1.16 \text{ min}^{-1}/\text{min}^{-1}$ (95CI between 1.01 and 1.27), which matched this expectation. In future studies, contrast agents with different average diameters should be tested to experimentally investigate the range of $R^{K^{trans}}$ that can be reliably measured, which can be compared with the simulated results in this report. Furthermore, DCE MRI has been used to measure relative differences in vascular permeabilities to assess differences in angiogenesis between tumors and longitudinal changes in a single tumor caused by anti-angiogenic chemotherapies (13). To assess these relative differences and changes in angiogenesis, the value of $R^{K^{trans}}$ should change from ~ 1 . Therefore the relative diameters of the two contrast agents should be different for these biomedical applications to measure a change in vascular permeability.

Other DCE MRI methods have used two relaxivity-based MRI contrast agents that were sequentially administered to a single animal, with a delay of minutes, hours, or days between injections of each agent. Yet changes in the hematocrit within tumor vascular physiology may occur between sequential injections, which will compromise a ratiometric comparison of the agents. Furthermore, rapid fluctuations in the hematocrit likely occur at the microscopic scale, which requires two agents to be simultaneously present to experience the same hematocrit in the same location in a tumor microvasculature (35). Therefore, DCE MRI results with sequential injections of contrast agents are inappropriate for analyses with the RAM. For comparison, the simultaneous injection and selective detection of two contrast agents is appropriate for the RAM, which we accomplished in this study by using two ^{19}F MRI contrast agents.

To our knowledge, our report demonstrates the first example of two ^{19}F MRI contrast agents that were selectively detected after co-injection during an in vivo study. Optimizing the selective excitation was critical for this demonstration. Attempts to implement selective detection after nonselective or selective excitation resulted in lower SNR, and therefore were not implemented in the final method. Optimizing a multi-echo detection method while considering the effects of J-modulation and T_2 relaxation were also critical for optimizing the SNR of images for each agent. For comparison, MR spectroscopic methods have been used to simultaneously detect two ^{19}F contrast agents in the same mouse, but not in the same tissue location (36,37).

The wide range of ^{19}F chemical shifts facilitated the selective detection of the two agents in this study. Similarly, the selective detection of two co-injected agents has also been accomplished using Chemical Exchange Saturation Transfer (CEST) MRI (38), which exploited a wide ~ 70 ppm range of ^1H chemical shifts generated by paramagnetic lanthanide chelates to selectively detect each agent. These CEST MRI studies used two polymer-based agents that had different average diameters, which showed different pharmacokinetic rates in tumor tissue. The RAM is ideally suited to quantitatively analyze DCE-CEST MRI results with two selectively detectable CEST agents.

The selective detection of two MRI contrast agents at the same time and same location has important implications for molecular imaging. As an analogy, selective detection of optical imaging contrast agents at different color wavelengths has revolutionized molecular evaluations during biochemical tests, in vitro studies, and ex vivo analyses. However,

optical imaging during in vivo studies is limited due to its limited spatial resolution and inability to interrogate deeper tissues. The selective detection of two or more MRI contrast agents such as the two ^{19}F nanoemulsions detected in this DCE MRI study, and quantitative analysis of two agents with models such as the RAM presented in this study, has strong potential to impact in vivo molecular imaging.

CONCLUSIONS

In conclusion, the RAM can accurately and precisely estimate relative vascular permeabilities of two contrast agents in a tumor. This model provides multiple advantages relative to traditional analyses of DCE MRI and advanced analysis of DCE MRI with the RRM. This study also demonstrated that two perfluorinated MRI contrast agents can be selectively detected in tumor tissue during a single DCE MRI scan session.

References

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med.* 1971; 285:1182–1186. [PubMed: 4938153]
2. Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature.* 1989; 339:58–61. [PubMed: 2469964]
3. Kaban K, Herbst RS. Angiogenesis as a target for cancer therapy. *Hematol Oncol Clin North Am.* 2002; 16:1125–1171. [PubMed: 12512387]
4. Taylor JS, Tofts PS, Port R, Evelhoch JL, Knopp M, Reddick WE, Runge VM, Mayr N. MR imaging of tumor microcirculation: promise for the new millennium. *J Magn Reson Imaging.* 1999; 10(6):903–907. [PubMed: 10581502]
5. Gillies R, Zafrani B, Guinebretiere JM, Meunier M, Lucidarme O, Tardivon AA, Rochard F, Vanel D, Neuenschwander S, Arriagada R. Ductal carcinoma in situ: MR imaging-histopathologic correlation. *Radiology.* 1995; 196:415–419. [PubMed: 7617854]
6. Daldrup H, Shames DM, Wendland M, Okuhata Y, Link TM, Rosenau W, Lu Y, Brasch RC. Correlation of dynamic contrast-enhanced MR imaging with histologic tumor grade: comparison of macromolecular and small molecular contrast media. *Am J Roentgenol.* 1998; 171:941–949. [PubMed: 9762973]
7. Jordan BF, Runquist M, Raghunand N, Gillies RJ, Tate WR, Powis G, Baker AF. The Thioredoxin-1 Inhibitor 1-Methylpropyl 2-Imidazolyl Disulfide (PX-12) Decreases Vascular Permeability in Tumor Xenografts Monitored by Dynamic Contrast Enhanced Magnetic Resonance Imaging. *Clin Cancer Res.* 2005; 11:529–36. [PubMed: 15701837]
8. Costouros NG, Lorang D, Zhang Y, Miller MS, Diehn FE, Hewitt SM, Knopp MV, Li KCP, Choyke PL, Alexander HR, Libutti SK. Microarray gene expression analysis of murine tumor heterogeneity defined by dynamic contrast-enhanced MRI. *Molec Imaging.* 2002; 1(3):301–308. [PubMed: 12920855]
9. Cooper RA, Carrington BM, Lancaster JA, Todd SM, Davidson SE, Logue JP, Luthra AD, Jones AP, Stratford I, Hunter RD, West CM. Tumour oxygenation levels correlate with dynamic contrast-enhanced magnetic resonance imaging parameters in carcinoma of the cervix. *Radiother Oncology.* 2000; 57(1):53–59.
10. Haider MA, Sitartchouk I, Roberts TPL, Fyles A, Hashmi AT, Milosevic M. Correlations between dynamic contrast-enhanced magnetic resonance imaging-derived measures of tumor microvasculature and interstitial fluid pressure in patients with cervical cancer. *J Magn Reson Imaging.* 2007; 25(1):153–159. [PubMed: 17173303]
11. Tunca F, Giles Y, Salmaslioglu A, Poyanli A, Yilmazbayhan D, Terzioğlu T, Tezelman S. The preoperative exclusion of thyroid carcinoma in multinodular goiter: Dynamic contrast-enhanced magnetic resonance imaging versus ultrasonography-guided fine-needle aspiration biopsy. *Surgery.* 2007; 142(6):992–1002. [PubMed: 18063087]
12. Evelhoch JL. Key factors in the acquisition of contrast kinetic data for oncology. *J Magn Reson Imaging.* 1999; 10:254–259. [PubMed: 10508284]

13. Cárdenas-Rodríguez J, Li Y, Galons JP, Cornell H, Gillies RJ, Pagel MD, Baker AF. Imaging biomarkers to monitor response to the hypoxia-activated prodrug TH-302 in the MiaPaCa2 flank xenograft model. *Magn Reson Imaging*. 2012; 30:1002–1009. [PubMed: 22554971]
14. Heisen M, Fan X, Buurman J, van Riel NA, Karczmar GS, ter Haar Romeny BM. The influence of temporal resolution in determining pharmacokinetic parameters from DCE-MRI data. *Magn Reson Med*. 2010; 63:811–816. [PubMed: 20187187]
15. Henderson E, Rutt BK, Lee T-Y. Temporal sampling requirements for the tracer kinetics modeling of breast disease. *Magn Reson Imaging*. 1998; 16:1057–1073. [PubMed: 9839990]
16. Dintenfass L. Hemorheology of cancer metastases: an example of malignant melanoma. Survival times and abnormality of blood viscosity factors. *Clin Hemorheol*. 1982; 2:259–271.
17. Barbee JH, Cokelet GR. The Fahraeus effect. *Microvasc Res*. 1971; 3(1):6–16. [PubMed: 5092929]
18. Fahraeus R, Lindqvist T. The viscosity of the blood in narrow capillary tubes. *Am J Physiol*. 1931; 96:562–8.
19. Tofts PS. Modeling tracer kinetics in dynamic Gd-DTPA MR imaging. *J Magn Reson Imaging*. 1997; 7:91–101. [PubMed: 9039598]
20. Roberts C, Hughes S, Naish JH, Holliday K, Watson Y, Cheung S, Buonaccorsi GA, Young H, Clarke N, Parker GJ. Use of An Individually Measured Hematocrit in DCE-MRI studies. *Proc Intl Soc Mag Reson Med*. 2011; 19:1078.
21. Just N, Koh DM, D'Arcy J, Collins DJ, Leach MO. A sssessment of the effect of haematocrit-dependent arterial input functions on the accuracy of pharmacokinetic parameters in dynamic contrast-enhanced MRI. *NMR Biomed*. 2011; 24(7):902–915. [PubMed: 21290457]
22. Strecker R, Scheffler K, Buchert M, Mross K, Dreves J, Hennig J. DCE-MRI in clinical trials: data acquisition techniques and analysis methods. *Int J Clin Pharmacology Therapeutics*. 2003; 41(12): 603–605.
23. O'Connor JPB, Jackson A, Parker GJM, Jayson GC. DCE-MRI biomarkers in the clinical evaluation of antiangiogenic and vascular disrupting agents. *Br J Cancer*. 2007; 96(2):189–195. [PubMed: 17211479]
24. Giraudeau C, Flament J, Marty B, Boumezbeur F, Meriaux S, Robic C, Port M, Tsapis N, Fattal E, Giacomini E, Lethimonnier F, Le Bihan D, Valette J. A New Paradigm for High- Sensitivity F-19 Magnetic Resonance Imaging of Perfluorooctylbromide. *Magn Reson Med*. 2010; 63:1119–24. [PubMed: 20373415]
25. Giraudeau C, Djemaï B, Ghaly MA, Boumezbeur F, Meriaux S, Robert P, Port M, Robic C, Bihan DL, Lethimonnier F, Valette J. High sensitivity 19F MRI of a perfluorooctyl bromide emulsion: application to a dynamic biodistribution study and oxygen tension mapping in the mouse liver and spleen. *NMR Biomed*. 2011; 25:654, 60. [PubMed: 21953998]
26. Tofts P, Brix G, Buckley D, Evelhoch J, Henderson E, Knopp M, Larsson H, Lee T, Mayr N, Parker G, Port R, Taylor J, Weisskoff R. Estimating kinetic parameters from dynamic contrast-enhanced T-1-weighted MRI of a diffusable tracer: Standardized quantities and symbols. *J Magn Reson Imaging*. 1999; 10:223–32. [PubMed: 10508281]
27. Yankeelov T, Luci J, Lepage M, Li R, Debusk L, Lin P, Price R, Gore J. Quantitative pharmacokinetic analysis of DCE-MRI data without an arterial input function: a reference region model. *Magn Reson Imaging*. 2005; 23:519–29. [PubMed: 15919597]
28. Jaspers K, Aerts H, Leiner T, Oostendorp M, van Riel N, Post M, Backes W. Reliability of pharmacokinetic parameters: Small vs. medium-sized contrast agents. *Magn Reson Med*. 2009; 62:779–87. [PubMed: 19623622]
29. Neubauer AM, Sim H, Winter PM, Caruthers SD, Williams TA, Robertson JD, Sept D, Lanza GM, Wickline SA. Nanoparticle Pharmacokinetic Profiling In Vivo Using Magnetic Resonance Imaging. *Magn Reson Med*. 2008; 60:1353–1361. [PubMed: 19025903]
30. Kassner A, Thornhill RE, Liu F, Winter PM, Caruthers SD, Wickline SA, Lanza GM. Assessment of tumor angiogenesis: dynamic contrast-enhanced MRI with paramagnetic nanoparticles compared with Gd-DTPA in a rabbit Vx-2 tumor model. *Contrast Media Mol Imaging*. 2010; 5:155–61. [PubMed: 20586031]

31. Fan X, River J, Muresan A, Popescu C, Zamora M, Culp R, Karczmar G. MRI of perfluorocarbon emulsion kinetics in rodent mammary tumours. *Phys Med Biol.* 2006; 51:211–20. [PubMed: 16394334]
32. Galbraith S, Lodge M, Taylor N, Rustin G, Bentzen S, Stirling J, Padhani A. Reproducibility of dynamic contrast-enhanced MRI in human muscle and tumors: comparison of quantitative and semi-quantitative analysis. *NMR Biomed.* 2002; 15:132–142. [PubMed: 11870909]
33. Haacke, EM.; Brown, RW.; Thompson, MR.; Venkatesan, R. *Magnetic Resonance Imaging: Physical Principles and Sequence Design.* New York: Wiley & Sons; 1999.
34. Kovar D, Lewis M, Karczmar G. A new method for imaging perfusion and contrast extraction fraction: Input functions derived from reference tissues. *J Magn Reson Imaging.* 1998; 8:1126–34. [PubMed: 9786152]
35. Kamoun WS, Chae S-S, Lacorre DA, Tyrrell JA, Mitre M, Gillissen MA, Fukumura D, Jain RK, Munn LL. Simultaneous measurement of RBC velocity, flux, hematocrit and shear rate in vascular networks. *Nature Methods.* 2010; 7(8):655–660. [PubMed: 20581828]
36. Yu JX, Kodibagkar VD, Liu L, Mason RP. A ^{19}F NMR Approach using Reporter Molecule Pairs to Assess β -Galactosidase in Human Xenograft Tumors *in Vivo*. *NMR Biomed.* 2008; 21(7):704–712. [PubMed: 18288788]
37. Partlow KC, Chen J, Brant JA, Neubauer AM, Meyerrose TE, Creer MH, Nolta JA, Caruthers SD, Lanza GM, Wickline SA. ^{19}F magnetic resonance imaging for stem/progenitor cell tracking with multiple unique perfluorocarbon nanobeacons. *FASEB J.* 2007; 21:1647–1654. [PubMed: 17284484]
38. Ali MM, Yoo B, Pagel MD. Tracking the relative in vivo pharmacokinetics of nanoparticles with PARACEST MRI. *Molec Pharmaceutics.* 2009; 6(5):1409–1416.

Fig. 1.

The simulated concentration-activity curves of the tumor for RAM. The pharmacokinetics of each contrast agent in the tumor were simulated using intervals of 1 sec and setting K^{trans} equal to $8.2 \times 10^{-3} \text{ min}^{-1}$ and $4.3 \times 10^{-3} \text{ min}^{-1}$ for CA-1 and CA-2, respectively. The AIF used for this simulation was based on Eq. [16] using experimental measurements of exponential decay rates ($\alpha = 16.54 \text{ min}^{-1}$, $\beta = -0.040 \text{ min}^{-1}$), and reported values for the distribution volumes ($A = 24.0 \text{ kgL}^{-1}$, $B = 5.5 \text{ kgL}^{-1}$) (26,27). The EES fractional volume (v_e) was set to 0.5 mL/mL for both curves (28).

Fig. 2.

Systematic errors as a function of temporal resolution. To estimate systematic errors in estimated $R^{K_{trans}}$, A) activity curves were simulated with $K^{trans,CA-1}$ ranging from 0.001 to 0.150 min^{-1} in steps of 0.003 min^{-1} while fixing $K^{trans,CA-2}$ at $4.3 \times 10^{-3} \text{ min}^{-1}$ and v_e at 0.5 mL/mL, or B) activity curves were simulated with v_e ranging from 0.10 to 0.9 mL/mL in steps of 0.005 mL/mL while fixing $K^{trans,CA-1}$ and $K^{trans,CA-2}$ at 8.2×10^{-3} and $4.3 \times 10^{-3} \text{ min}^{-1}$, respectively. These curves were down-sampled to 0.5, 2, and 4 min temporal resolutions. C, D) The same procedure was used to estimate systematic errors in estimated $k_{ep,CA-1}$.

Fig. 3.

Random errors as a function of signal-to-noise ratio and temporal resolution. Simulated activity curves were down-sampled to 0.5, 2, and 4 min temporal resolutions. Gaussian noise was added to produce SNRs from 5 to 100 for 1000 data sets. A) The accuracies of estimating R^{Ktrans} are shown by the data symbols, and the precisions of the R^{Ktrans} estimates are shown by the error bars that represent the standard deviation of 1000 trials. B) The accuracies and precisions of estimating $k_{ep,CA-1}$ are also shown, with a y-axis that spans a range that is approximately 10-fold greater than the range of the y-axis in Figure 3A to highlight the greater error in estimating $k_{ep,CA-1}$.

Fig. 4. ^{19}F MR signals. A) A MR spectrum shows a single resonance for PCE and a single resonance for the fluoromethyl group of PFO. The fluoromethylene groups of PFO have multiple resonances, but these resonances can be neglected when PCE and the fluoromethyl group of PFO are selectively detected. B) The MR imaging signal from PCE was linearly related to ^{19}F concentration of the sample. A spin-echo MRI protocol was used at 37°C . Error bars represent the standard deviation of signal values from pixels of the image of each phantom. C) MR images of phantoms consisting of 100% PCE, 100% PFO, and a 50:50 v/v mixture of PFO and PCE were acquired with selective detections of PCE and PFO, and with no selective detection.

Fig. 5.

The effect of J-modulation on T_2 relaxation of the ^{19}F nanoemulsions. The effective echo time is equal to the number of echoes multiplied by TE. A) The T_2 relaxation of the ^{19}F MRI signal of PCE is not affected by TE. B) The T_2 relaxation of the ^{19}F MRI signal of PFO is affected by TE due to J-modulation caused by the fluoromethylene groups. C) PCE and PFO have equal T_2 values when TE equals 25 msec.

Fig. 6.

The experimental AIF of PCE. Four mice with subcutaneous MDA-MB-231 tumors were injected with 150 μL of a 40% v/v nanoemulsion of PCE. A) The average ^{19}F MRI signal and B) the average ^{19}F MRI SNR was monitored in C) a 2x2 voxel region in the tail that represented a tail vein (highlighted in green). The noise level was 1.0 relative to the MRI signal scale that was used for this analysis, so that the signal and SNR were identical at each time point. Error bars represent the standard deviation of the measurements of four mice. The signal activity curve shown as a solid red line was fitted with a biexponential model (Eq. [15]).

Fig. 7. Selective detection of two ¹⁹F nanoemulsions during an in vivo MRI study. A) The ¹⁹F DCE MRI results of a mouse model with a MDA-MB-231 tumor after co-injection of PCE and PFO showed accumulation of each agent in the tumor tissue. B) A ¹H MR image provides an anatomical reference for the ¹⁹F MRI results, which demonstrates that a region of the tumor showed substantial ¹⁹F MR signal. C) The ¹⁹F concentrations of PCE and PFO show a similar temporal dependence in tumor tissue. Based on the dynamic changes in concentration of PCE and PFO in tumor tissue, the R^{Ktrans} of the tumor tissue was determined to be 1.16 min^{-1} , $k_{ep,CA-1}$ was $9.1 \times 10^{-3} \text{ min}^{-1}$, and the root mean square error of the fitting was $2.7 \times 10^{-2} \text{ mM}$.