Towards absolute quantification of perfusion using
dynamic, susceptibility-weighted, contrast-enhanced
(DSC) MRI

by

Vishal Patil

A dissertation submitted in partial fulfillment
Of the requirements for the degree of
Doctor of Philosophy
Department of Basic Medical Sciences
Center for Biomedical Imaging
New York University
May, 2013

__________________________________________
Glyn Johnson Ph.D.
DEDICATION

To Mom – you are the strongest person I know
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my thesis committee: Drs. Dan Turnbull, Gene Kim, Eric Sigmund and Roman Fleysher. The quality of my thesis and its direction are a product of their expert insights. It is evident from the amount of time they spent reflecting on this document how much care and pride they take in mentoring their students. I feel very fortunate to have been in their presence during my time as a graduate student. I also want to thank those that have mentored and helped me publish papers, Drs. Jens H. Jensen and Ivan Kirov. Of course, thanks to those in room 420 and CBI who have made my time in the office most enjoyable.

The encouragement to pursue a PhD came from two faculty members during my undergraduate studies at Purdue University. Dr. Rob Stewart was the best lecturer I ever had. After completing his Radiation Science Fundamentals course in the fall of 2005, I knew I wanted explore the field of medical physics. He introduced me to Dr. Jian Jian Li and the following week I started conducting independent scientific research. I have not stopped since. Dr. Li taught me so much about research and how to think like a scientist. I cannot describe how grateful I am to him for taking me on as an undergraduate research assistant and giving me the quality of education I needed to be successful moving forward.
I could not have gone through the grind without my closest friends here at NYU. Garrett and Caleb – even though smart money should have been against us, it is only fitting we start and end our PhDs together. You two will always be my brothers. Gene – I could not have kept sane without all those rounds of golf. And thanks for being my Eurotrip travel buddy. Erik – my roomie, I cannot wait to attend your thesis defense. Thanks for always being there for me.

Finally, and most importantly, thanks to Dr. Glyn Johnson who has mentored me throughout my graduate studies. Our time together was perfect. I thank you for everything you have done for me – the papers we published, the freedom to pursue projects I thought were interesting, the moral support when things were not looking good, and of course, the rare pat on the back. I will never forget all of those hours spent in your office talking about everything but science. You letting me get to know you as a person was the best thing you have ever given me. I am forever in your debt.
ABSTRACT

Measuring perfusion in brain tissue is important for characterizing and assessing neuronal physiology in vascular neurological diseases. Studies have shown estimates of cerebral blood volume (CBV) and flow (CBF) are able to grade tumors and determine a prognosis in patients suffering from cancer and stroke. One powerful tool able to estimate perfusion is contrast based magnetic resonance imaging (MRI), the most common application being dynamic, susceptibility-weighted, contrast-enhanced (DSC) MRI. Generally, DSC MRI is performed by acquiring a series of images during the time course of an intravenously administered contrast agent. CBV and CBF quantification depend on estimating contrast agent concentration from the signal-time series and then applying the intravascular-indicator dilution theory.

Although abnormalities can be visualized by hyper or hypo-intensities in signal relative to normal appearing tissue, leading to relative perfusion measurements, the ability to characterize pathological tissue in a reliable manner depends on the accuracy of absolute perfusion measurements. Unfortunately, absolute quantification of CBV and CBF are difficult to make and irreproducible due to imaging limitations and simplistic assumptions; the most detrimental being assuming a linear relaxivity – the relationship between signal and contrast agent
concentration. Most all DSC MRI studies, whether technical or clinically related, have assumed linearity because the true relationship in tissue and venous blood \textit{in vivo} are unknown.

The purpose of this thesis is to: 1.) Provide an overview of intravascular-indicator dilution theory and quantitative DSC MRI. 2.) Present an analytical function used to model DSC MRI time series. 3.) Investigate the errors associated with assuming linearity. 4.) Determine reliable nonlinear relaxivity functions for tissue and venous blood. 5.) Quantify perfusion measurements in a clinical study using nonlinear relaxivity functions. 6.) Test the accuracy of a new imaging method called magnetic field correlation imaging to estimate contrast agent concentration \textit{in vitro}. 
# TABLE OF CONTENTS

DEDICATION ........................................................................................................................................ iv

ACKNOWLEDGEMENTS ................................................................................................................... v

ABSTRACT ........................................................................................................................................ vii

LIST OF FIGURES .............................................................................................................................. xii

LIST OF TABLES ................................................................................................................................. xvii

LIST OF APPENDICES ...................................................................................................................... xviii

INTRODUCTION ................................................................................................................................. 1

  A Brief History of Measuring Perfusion ..................................................................................... 1

  Perfusion Imaging with MRI ...................................................................................................... 6

CHAPTER 1: On the Theory of Measuring Perfusion ................................................................. 11

  The Central Volume Theorem ................................................................................................. 11

  Applying the Intravascular-Indication Dilution Theory to DSC MRI ................................ 12

CHAPTER 2: Fundamentals of Quantitative DSC MRI ............................................................... 16

  Image Acquisition ...................................................................................................................... 16

  Signal Theory .............................................................................................................................. 16

  Deconvolution ............................................................................................................................ 18

  Sources of Quantification Error ............................................................................................... 21

CHAPTER 3: The Single Compartment Recirculation Model .................................................. 23
LIST OF FIGURES

Fig. I.1 – Portrait of G.N. Stewart..........................................................................................5
Fig. I.2 – Portraits of S.S. Kety (left) and C.F. Schmidt (right)........................................5
Fig. I.3 – Portraits of F. Bloch (top left), E. Purcell (top right), P. Lauterbur (bottom left) and Sir P. Mansfield (bottom right).................................................................10
Fig. 3.1 – Diagram of a typical signal time curve illustrating how the last fitted point for the gamma variate was selected for each method: i) third point after the bolus minimum, ii) point of the half drop of the bolus after the bolus minimum, iii) the point after the minimum at which the signal exceeds the post bolus signal minus the standard deviation of the pre bolus signal ($\sigma$) and iv) by visual determination. ........................................................................................................................................29
Fig. 3.2 – Example of the bolus (solid line) and recirculation (dotted line) portions of the SCR model and bolus (dashed line) of a GV fitted to a typical concentration time curve (crosses)..................................................................................................................................................30
Fig. 3.3 – Fits (line) to concentration time curves (crosses). The left column gives the “noiseless” concentration time curves and the right has added noise to give an SNR of 25.............................................................................................................................................32
Fig. 3.4 – Percent error (a) and percent deviation (b) of the area under the curve (AUC) plotted against SNR for each model. Percent error (c) and percent deviation (d) of the first moment (FM) plotted against SNR for each model..........................33

Fig. 4.1 – $T_2^*$-weighted DSC MRI image acquired at 3 T. Region of interests and single pixels were drawn to measure signal intensities in, WM, normal appearing white matter; and GM, caudate nucleus.................................................................51

Fig. 4.2 – Plots of measured (+) and fitted values of log ratio, $\lambda$. Each plot is the concatenation of 5 data sets at 3 T, $T_E = 32$ ms. a) Linear formulation. b) Nonlinear formulation.................................................................52

Fig. 4.3 – Plots of $\Lambda$ vs. $C$ for the optimized linear (dashed) and SDR (solid) formulations ($B_0 = 3$ T; $T_E = 32$ ms). ........................................................................................................52

Fig. 4.4 – Plots of estimated blood contrast agent concentration from a single patient at a) 1.5 T and b) 3 T. The concentration estimates were obtained from signals in arteries (red line), white matter using the linear (blue line) and nonlinear (green line) formulations. The arterial and white matter concentrations calculated using the nonlinear formulation is in excellent agreement when considering delay and dispersion.................................................................53

Fig. 4.5 – Box and whisker plots of fractional error in ROI and single pixel measurements in white matter (a and b) and grey matter (c and d) CBV estimates obtained using the linear and nonlinear formulations at 1.5 T and 3 T. In each box, the central line represents the median of measurements in five test patients, the
upper and lower boundaries of the box represent the upper and lower quartiles and the whiskers represents the range..........................................................54

Fig. 4.6 – Blood concentration-time curves in WM (blue) and GM (green) from a single test patient calculated using linear and non-linear formulations at 1.5 T (Figs. 4.6a and e) and 3 T (Figs. 4.6c and g), respectively. Differences between scaled GM and WM curves from the beginning of the bolus to 30 seconds after are plotted against WM concentration over all test patients at using linear and non-linear formulations at 1.5 T (Fig. 4.6b and f) and 3 T (Fig. 4.6d and f), respectively. ......56

Fig. 5.1 – Measured (points) and fitted (solid lines) venous signal intensity time curves from five patients at 1.5T (a) and 3 T (b). The fitted curves were calculated using the relaxivity parameters that gave the best agreement between the area under the bolus, and steady state amplitude of arterial and venous concentration time curves. ..........................................................71

Fig. 5.2 – Arterial (red) and venous (in vitro – black; in vivo – blue) relaxivity calibration curves at 1.5 T (a) and 3 T (b). Curve coefficients are given in Table 5.1. .........................................................................................72

Fig. 5.3 – Arterial and venous CTCs at 1.5T (a) and 3T (b). The discontinuities in the in vitro venous curves are because the same value of $\Delta R_2^*$ corresponds to two different concentrations below about 2 mM (see Fig. 5.2). The curve was therefore obtained by interpolation between zero and the first unambiguous value of $\Delta R_2^*$..73
Fig. 6.1 – Perfusion parameters for the different subject groups measured pre- (deep gray) and post-treatment (light grey). White and grey matter measurements are given in the top and bottom rows respectively. Statistically significant differences are indicated by asterisks (p values can be found in Table 6.2).

Fig. 6.2 – Fractional rates of change of each parameter per month (i.e., the vertical scale is in units of month\(^{-1}\)) for CBF (left), CBV (middle) and MTT (right) for white (top) and grey (bottom) matter. Rates of change that are significantly non-zero are marked by an asterisk (p values can be found in Table 6.3).

Fig. 7.1 – Cell suspension signal intensities with 4 mM Gd-DTPA versus a: the MFC refocusing pulse shift (\(|t_r| \leq 8\) ms), b: \(T_1\) in \(T_1\) measurements (\(\leq 1000\) ms), c: \(T_E\) in \(T_2\) measurements (\(T_E \leq 67\) ms), and d: \(T_E\) in \(T_2^*\) measurements (\(T_E \leq 60\) ms). The lines are fits to Eq. (7.6) (a), Eq. (7.7) (b) and Eq. (7.8) (c, d). Standard error estimates for all data points are shown with error bars.

Fig. 7.2 – MFC (a) and relaxation rates \(R_1\) (b), \(R_2\) (c) and \(R_2^*\) (d) plotted against Gd-DTPA concentration for cell suspensions and water phantoms. MFC in the cell suspensions is fitted to a quadratic polynomial. In the relaxation rate plots, solid and dashed lines represent a linear extrapolations, based on the \([\text{Gd}] = 0\) and \([\text{Gd}] = 1\) mM data points for the cell suspension and water phantom respectively. Standard error estimates for all data points are shown with error bars.

Fig. 7.3 – Plot of MFC difference as a function of Gd-DTPA concentration (Eq. (7.4)).
Fig. 7.4 – Bland-Altman plots for MFC (a) and relaxation rates $R_1$ (b), $R_2$ (c) and $R_2^*$ (d). The y axis represents the difference and the x axis the mean of estimated and true Gd concentrations respectively. The solid line indicates the mean difference and the dashed lines show ± 95% limit agreements (i.e., approximately twice the standard deviation of the difference).
LIST OF TABLES

Table 4.1 – Fractional error in white and grey matter CBV estimates (%) relative to literature values by the linear and nonlinear equations at 1.5 and 3T for both ROI and single voxel measurements..........................................................................................................................55

Table 5.1 – Relaxivity coefficients for Eq. (5.3) (curves shown in Fig. 5.2)...............72

Table 6.1 – Patient demographics for each group.....................................................83

Table 6.2 – The values of each perfusion measure (Mean ± SD) at the last time point pre-treatment and the first time point post-treatment; the change in value and the p value from a paired sample t test. Units are as follows: CBF, ml/100 g/min; CBV, ml/100g; MTT, s. Statistically significant changes are shown in bold...........84

Table 6.3 – Fractional rates of change of each parameter per month in serial measurements. Statistically significant results are shown in bold. CBV declined at a statistically significant rate after treatment with bevacizumab. No other rates of change were significantly different from zero. Note that pre-treatment rates of change were only available in recurrent glioma patients, Group III, since only a single initial pre-treatment scan was available for each newly diagnosed patient...86
LIST OF APPENDICES

APPENDIX A ........................................................................................................108
APPENDIX B ........................................................................................................111
APPENDIX C ........................................................................................................113
INTRODUCTION

A Brief History on Measuring Perfusion

In 1897 G.N. Stewart attempted to develop a method to measure cardiac output applicable to animals of any size (1). Stewart’s motivation came from the large discrepancies in estimates between previous studies dating back to 1850. Before Stewart’s study, measuring cardiac output in animals (rabbits, dogs and horses) employed rather crude methods. Direct measurements involved cutting off circulation by ligature and measuring the amount of blood passing through the system (2) or inserting a rheometer in the aorta (3). Indirect measurements estimated the amount of oxygen added to blood as it passed through the lungs (4) using the Fick principle – blood flow is proportional to the difference in concentration of a substance in blood as it enters and leaves an organ (5). Stewart had the idea of injecting an indicator (sodium chloride) directly into an anaesthetized dog, taking samples of blood before and after injection during crucial time points, and then quantifying the amount of indicator in each sample by the Kohlrausch method – an ingenious method which measures electrolyte resistance in a solution by a Wheatstone bridge connected to a telephone. Stewart’s groundbreaking paper paved the way for thousands of future studies measuring perfusion by means of an indicator.
The first study measuring blood flow in the human brain was conducted by S.S. Kety and C.F. Schmidt in 1945 (6). Subjects were instructed to inhale a mixture of gas consisting of 15% nitrous oxide and 85% oxygen through an anesthesia mask for 20 minutes. Nitrous oxide was used because it is physiologically inert, capable of diffusing across the blood-brain barrier and quantifiable in blood. During 2, 4, 6, and 10 minute intervals samples of arterial and venous blood were taken from the femur and right internal jugular vein, respectively, and the volume of nitrous oxide in the samples are quantified. Then finally cerebral blood flow was calculated using the Fick principle.

In the early 1960s regional cerebral blood flow measurements could be made using radioactive indicators. In 1961 N.A. Lassen and D.H. Ingvar injected a saline and krypton\textsuperscript{85} solution into cats to detect low energy beta particles with a Geiger-Muller tube (cats underwent a craniotomy and resection of the dura before injection). One of the first studies to measure cerebral blood flow with nuclear medicine in humans was conducted by D.H. Ingvar et al. in 1965 (7). Xenon\textsuperscript{133} was injected into the internal carotid artery and the emitted gamma-rays are measured externally using a scintillation detector around four regions of the brain. Although using radioactive isotopes to measure perfusion invasively has its advantages i.e. it is inexpensive and easy to conduct, there are a number of disadvantages both in methodology and quantification. The topographical detection field cannot be precisely defined which may result in overlapping counts between regions.
Quantitatively, clearance curves exhibit multi-exponentiality making it difficult to determine an accurate extraction curve (8).

Invasive methods started giving way to noninvasive methods for sampling indicator, and with the advent of proton emission tomography (PET) by D.E. Kuhl and colleagues in the 1960s (9), cerebral blood flow measurements could be quantified from images which displayed indicator concentration levels. PET imaging is conducted by intravenously introducing a diffusable radioactive indicator which is converted into a biologically useful molecule, waiting a short period of time (~2-120 min) until the tracer has reached a substantial concentration in the tissue of interest and then imaging to measure the indicator concentration as it decays.

PET provides important clinical information regarding metabolic functionality and is still considered the “gold standard” for measuring blood flow, but there are a number of disadvantages. The radioactive indicators may be dangerous to patients who are sensitive to radiation – it is generally advised for women who are pregnant or breast feeding to avoid PET scans. Compared to other imaging modalities, PET is expensive and provides low spatial resolution. Furthermore, a typical PET scan lasts between ~45-60 min making it uncomfortable for the patient who is expected to be lying still.

Another imaging modality able to measure perfusion is computerized tomography (CT). CT was first developed by G.N. Hounsfield in 1973 (10), and a well formulated theory for estimating perfusion via CT was presented by L. Axel in
By the early 1990s, perfusion measurements could be estimated using conventional CT scanners, and with the introduction of fast multi-detector CT in the late 1990s, measuring perfusion via CT became a practical clinical technique. Perfusion CT is typically conducted by administering an iodinated contrast agent (CA) intravenously and dynamically imaging the anatomy of interest as the first-pass moves through the vasculature.

The main advantages of perfusion CT: 1) wide availability of scanners and commercial perfusion analysis software. 2) Simplicity of contrast enhancement quantification – iodinated contrast medium has a linear relationship with X-ray attenuation. 3) Repeatability and relatively low inter-operator variability. Of course, the main disadvantage of CT (or any technique dependent on ionizing radiation) is radiation exposure to the patients. This can be further exacerbated by attempting to increase the already limited anatomical coverage.
Fig. I.1 – Portrait of G.N. Stewart.

Fig. I.2 – Portraits of S.S. Kety (left) and C.F. Schmidt (right).
Perfusion Imaging with MRI

The basis of magnetic resonance imaging (MRI) is nuclear magnetic resonance (NMR) which was first described by F. Bloch and E. Purcell in 1946. Both won the 1952 Nobel Prize for Physics. Bloch and Purcell independently found that certain atoms absorbed energy in the radiofrequency (RF) range when placed in a magnetic field and emanated this same energy when returning back to their original energy state. In 1973 the physics of NMR was adapted by P. Lauterbur and Sir P. Mansfield, the inventors of what we know to be MRI, and were awarded the 2003 Nobel Prize for Physiology or Medicine.

In short, MRI works by exploiting the unique magnetic properties of the body’s tissue to create high resolution images without the use of radiation. When a sample abundant in water is placed in a high magnetic field (on the order of $10^5$ times stronger than the Earth’s), the hydrogen nuclei align along the direction of the external magnetic field. RF energy tuned to the exact resonating frequencies of the nuclei is deposited in the sample – spatial resolution is obtained by altering the strength of local magnetic fields using gradient coils. Once the transmitting RF energy source is turned off, the affected nuclei emit RF energy while returning to a resting state. The emitted energy is detected by receiver coils placed around the sample and is used to construct MRI images. The time it takes for the nuclei to return to a resting state is termed “relaxation rate” and is measured in two components, longitudinal and transverse. Because tissues have distinct intrinsic
relaxation rates, the imaging protocol can be altered to emphasize a particular tissue in an MRI image.

There are two main types of techniques for measuring perfusion using MRI, one which utilizes an endogenous and another which uses exogenous indicator. Arterial spin labeling (ASL), uses labeled blood as an endogenous indicator and was first introduced by Williams et al. in 1992 (13). A general overview of the method is as follows: First, water molecules in arterial blood which feeds the tissue of interest are labeled by inverting their magnetization. Next, an image is acquired as the labeled molecules enter the tissue of interest and then subtracted from another image of the same tissue of interest but without the labeled molecules. Since the absolute signal intensity difference is small, the experiment is repeated multiple times and perfusion maps are calculated using equations which relate cerebral blood flow to changes in magnetization. Many derivatives of the initial ASL method have been proposed and researchers continue to improve the technique especially with higher field strength scanners. Despite the advancements in technique and use of an endogenous indicator, ASL is not routinely used in the clinic. This is because of the low level of perfusion signal (approximately 2% of the raw signal) compared to the noise levels due to the rapid decay of labeled molecules. The high noise levels produce large random errors in the blood flow calculations (14).

The two methods which uses an exogenous indicator are dynamic, contrast-enhanced (DCE) and dynamic, susceptibility-weighted, contrast-enhanced (DSC)
MRI. The main advantages of contrast based MRI versus ASL is a substantially higher signal-to-noise (20-200) and ability to image a larger portion of the brain. Both contrast based MRI methods are typically performed by acquiring a series of images ($T_1$ for DCE and $T_2^*$ for DSC) before, during and after an injection of a paramagnetic CA called, gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA). The series of images are used to construct signal-time curves and then are transformed into concentration-time curves which are used in both DCE and DSC MRI analyses. DCE data is mainly used for pharmacokinetic modeling (calculating vasculature permeability and $K^{trans}$, the volume transfer constant between blood and extracellular-extravascular space) while DSC data is used in first-pass perfusion analysis to calculate absolute and relative cerebral blood volume (CBV), flow (CBF), and mean transit time (MTT) – the average time the CA remains in the tissue. DSC MRI will be the focus of this thesis.

DSC MRI was first developed in the late 1980s and early 90s by Rosen et al. (15) and is used to assess the hemodynamics of patients suffering from brain tumors (16-18), ischemia (19,20) and multiple sclerosis (19,21). Previous studies have shown that perfusion measurements made via DSC MRI can be used to grade tumors (22), quantitatively assess the degree of angiogenesis (23), guide directed biopsy (24) and evaluate the efficacy of anti-cancer therapies (25).

There are five main steps when performing a quantitative DSC MRI experiment.
1) Acquiring a series of images with a temporal resolution on the order of approximately 1 second before, during, and after a bolus of contrast agent is administered intravenously. Scan times usually range from 1-3 minutes and the first-pass of the contrast agent is the primary focus of analysis.

2) Making signal measurements to produce signal-time curves in an artery and in tissue which is further discussed in chapter 2.

3) Converting signal measurements to estimates of contrast agent concentration. Inaccurate estimates of concentration propagate throughout the entire subsequent analysis and are arguably the largest source of error. This step is the main focus of this thesis.

4) Modeling the first-pass by an analytical function to smooth the raw data, quantify the amount tracer injected and to reduce the effects of contrast agent leakage and recirculation (these effects, if great, can invalidate analysis). This is the subject of chapter 3. Modeling the first-pass simplifies the final step.

5) Calculating perfusion parameters. The equations necessary for calculating absolute CBV, CBF and MTT will be presented in chapter 1.

A major issue with quantitative DSC MRI is the poor reproducibility and accuracy of perfusion measurements in tissue. Lack of reproducibility has obvious consequences for the sensitivity and specificity for DSC based diagnoses. Lack of accuracy prevents meaningful comparison of results from different modalities e.g. PET or CT, and between MRI studies using different imaging protocols. For
example, the optimal rCBV cutoff for distinguishing low and high grade gliomas has been reported as anywhere between 1.5 and 5.6 (26-30).

Therefore, the overarching aim of the work presented in this thesis is to improve the reproducibility and accuracy of DSC methods.

Fig. I.3 – Portraits of F. Bloch (top left), E. Purcell (top right)†, P. Lauterbur (bottom left) and Sir P. Mansfield (bottom right)‡.

† “The Nobel Prize in Physics 1952”. Nobelprize.org 9 Apr 2013  
http://www.nobelprize.org/nobel_prizes/physics/laureates/1952/

‡ “The Nobel Prize in Physiology or Medicine 2003”. Nobelprize.org 9 Apr 2013  
CHAPTER 1: On the Theory of Measuring Perfusion

The Central Volume Theorem

It took twenty-four years after G.N. Stewart’s 1897 study when he realized that no study had measured the minute volume in the heart, \( V \), volume of blood in the lungs, \( Q \), and the mean pulmonary circulation time, \( M \), in the same animal. In 1921, G.N. Stewart achieved the aforementioned and determined that if two of the quantities are known then by the following relationship,

\[
V = QM
\]  

the third can be calculated (31).

As more sophisticated injection techniques developed, so did the understanding of the mathematical basis of indicator-dilution methods. In 1931, W.F. Hamilton et al. conducted experiments on humans which involved injecting an indicator (dye) and then using a 19 G. needle to collect serial samples of blood every 1-2 seconds from the femoral artery. The concentration of dye was determined colorimetrically, plotted against time and then a smooth curve was fit by hand through the data points thus giving rise to a concentration-time curve (CTC). From the CTC, flow, \( F \), and mean circulation time, \( M \), can be calculated

\[
F = \frac{m}{ct} \quad M = \sum \frac{c_i t_i}{c_n}
\]  

(1.2)
where \( m \) is the mass of dye, \( c \) is the average concentration during the first bolus pass, \( t \) is the time during the first bolus pass and \( c_n \) and \( t_n \) are the concentration and times at the \( n \)th reading, respectively. The volume of the system, \( V \), can then be calculated using what is known as the central volume theorem:

\[
V = FM
\]

(1.3)

A proof of the central volume theorem can be found in Appendix A.

**Applying the Intravascular Indicator Dilution Theory to DSC MRI**

In 1954 P. Meier and K.L. Zierler provided the first direct mathematical proof of Eq. (1.3) considering both an instantaneous and constant fusion under the following assumptions which make up the intravascular indicator dilution theory (32). 1.) Stationarity of flow – indicator entering a system will be dispersed exiting the system in the same manner as indicator entering the system at any other time. This assumption will be violated in systems where volume and flow change phasically, like the heart. 2.) Flow of indicator in a system is representative of the flow of the fluid in the system. 3.) There are no stagnant pools in the system. 4.) Recirculation is not present. It is apparent that depending on the injection protocol, some of the aforementioned assumptions will be violated. If the injection is instantaneous and the system displays non-stationarity properties then the injection time and phasic cycle must be taken into account which may lead to unreliable measurements. However, measurements may be insensitive using a constant infusion in a non-stationary system. Conversely, depending on the length of the
system, recirculation effects may be introduced in measurements if the injection is constant (i.e. constant infusion); in which case perhaps an instantaneous injection would be most advantageous.

The central equation for a DSC MRI experiment is

\[ C_i(t) = \frac{k_H}{\rho} FC_a(t) \otimes R(t) \]  

(1.4)

where \( C_a(t) \) is contrast agent concentration estimated from an artery feeding a tissue of interest, \( C_i(t) \). \( k_H \) is a constant which accounts for the difference between arterial and capillary hematocrit, \( \rho \) is the tissue density, \( \otimes \) denotes convolution, \( F \) is CBF and \( R(t) \) is the residue function to be defined below. It should be noted that in much of the literature \( \rho \) is ignored since it is very close to unity. However, it is necessary for Eq. (1.4) to be dimensionally correct.

Eq. (1.4) can be derived by the following equations. The volume of blood (i.e. CBV), \( v_b \), entering a volume of tissue, \( V_t \), in time \( d\tau \) is

\[ v_b = \frac{k_H}{\rho} F V_t d\tau \]  

(1.5)

where \( F \) is in standard units of mL/100 g of tissue/min. The quantity of indicator, \( q \), entering \( V_t \) at time \( \tau \) is then

\[ dq = \frac{k_H}{\rho} FC_a(\tau) V_t d\tau \]  

(1.6)
and the amount of indicator remaining at time \( t \), by the definition of \( R(t) \) – the residue function, is

\[
dq(t) = dq(\tau)R(t - \tau).
\]

(1.7)

Thus, the total quantity of indicator at time \( t \) is then obtained by integrating Eq. (1.7) between \( \tau = 0 \) and \( t \)

\[
q = \frac{kn}{\rho}FV\int_{0}^{t} C_a(\tau)R(t - \tau) d\tau,
\]

(1.8)

and can be rewritten as a convolution

\[
C_i(t) = \frac{q}{V_i} = \frac{kn}{\rho}FC_a(t) \otimes R(t).
\]

(1.9)

\( F \) and \( R(t) \) can be calculated by deconvolution methods discussed in the next chapter.

To calculate the mean transit time, MTT in standard units of s (i.e. \( M \) in the previous section), it can be proven that

\[
MTT = \int_{0}^{\infty} R(t) dt.
\]

(1.10)

A proof of Eq. (1.10) can be found in Appendix B.

The central volume theorem (Eq. (1.3)) can now be written in terms of absolute DSC MRI perfusion measurements to calculate CBV:

\[
CBV = CBF \cdot MTT.
\]

(1.11)

Alternatively, CBV can be calculated in standard units of mL/100 g of tissue using the following equation,
\[ CBV = \frac{k_{ll}}{\rho} \int \frac{C_t(t)dt}{\int C_a(t)dt} \] (1.12)

A proof of Eq. (1.12) can also be found in Appendix B.
CHAPTER 2: Fundamentals of Quantitative DSC MRI

Image Acquisition

Images for a DSC MRI experiment can be acquired using single or multi-shot gradient or spin echo echo planar imaging (EPI) – the vast majority of DSC MRI studies are performed using single-shot gradient echo EPI to create $T_2^*$-weighted images and will only be considered throughout this document. These pulse sequences are able to produce temporal resolutions (i.e. the repetition time, $T_R$) of ~1 s, are able to cover 5-12 slices with a matrix size of 128x128 and a resolution of ~1.8x1.8 mm$^2$. The echo time, $T_E$, ranges from 30-50 ms and the flip angle, $FA$, ranges from 30-90° with a slice thickness of 3-5 mm. Gd-DTPA is injected at a dose of 0.1-0.2 mmol/kg at a rate of 5 mL/s followed by a 10 mL bolus of saline to flush any residual contrast agent in the bloodstream. Typically, the total image acquisition time is 1-3 min and starts ~10 s before the bolus arrival time.

Signal Theory

The essential first step in quantitative DSC MRI is the conversion of signal intensity as a function of time, $S(t)$, to Gd-DTPA concentration,$C(t)$, and are related by the following equation
\[ S(t) = S_0 \exp\left(-\Delta R_2^* (t) T_E \right) \frac{\sin (FA) \left(1 - \exp\left( - R_t (t) T_E \right) \right)}{1 - \cos (FA) \exp\left( - R_t (t) T_E \right)} ; \]
\[ R_t (t) = R_t (0) + r^s C \]
\[ \Delta R_2^* (t) = r^s C (t) \]

where \( S_0 \) is a constant describing proton density and scanner gain, \( \Delta R_2^* (t) \) is the change in transverse relaxation rate \( (R_2^* \equiv 1/T_2^* ) \) due to the presence of \( C (t) \), \( r^s \) is a proportionality constant (a.k.a. the relaxivity constant) and \( r_t \) is the relaxivity constant used to relate \( C(t) \) to longitudinal relaxation rate, \( R_t (R_t \equiv 1/T_t) \).

Fortunately, \( T_t \) effects can be ignored if imaging parameters, such as \( FA \) and \( T_E \), are chosen to minimize \( T_t \) sensitivity (reducing \( FA \) and increasing \( T_E \)).

Dropping the \( T_t \) contribution from the equation above and substituting \( \Lambda (C) \), a unitless function describing the change in \( T_2^* \) due to the presence of CA, for \( \Delta R_2^* T_E \) yields,

\[ S(t) = S_{pre} \exp\left( -\Lambda (C) \right) \]

where \( S_{pre} \) is the average signal intensity before the bolus arrives. Eq. (2.1) is considered the fundamental signal equation for a DSC MRI experiment.

The transverse relaxation rate \( (T_2^* ) \) is affected by two processes: microscopic dipole-dipole interactions and “mesoscopic” interactions due to magnetic field perturbations caused by tissue structures in close proximity with differing susceptibilities. The mesoscopic effects are determined by compartmentalization of Gd-DTPA (that is to say CA in blood vessels) which creates susceptibility gradients between the vessel and surrounding tissue. Water
molecules lose phase coherence as they diffuse through these manufactured
gradients, resulting in shorter $T_2^*$ and in turn a decrease in signal intensity (33-35).

Before any DSC MRI analysis, signal measurements from arterial and tissue
must be made. Generally, arterial voxels are detected either manually or
automatically. Manual detection is conducted by an experienced researcher or
radiologist who determines individual signal-time series curves which appear to fit
the profile of an arterial voxel (large signal drop, early bolus arrival and narrow
bolus). Automatic detection entails implementing an algorithm which screens each
voxel and selects the likeliest arterial candidates (36-38). In some instances,
multiple arterial signals are averaged to increase SNR. Tissue measurements are
made by drawing regions of interest (ROI) around a tissue of interest. ROIs
encompass ~10 voxels and are averaged. Alternatively analysis may be performed
on every voxel to create perfusion maps. Once signal measurements are made, Eq.
(2.1) can be used to calculate $C_a(t)$ and $C_t(t)$, and finally CBV, CBF and MTT
can be calculated.

**Deconvolution**

Eq. (1.4), $C_i(t) = \frac{k_w}{\rho} FC_a(t) \otimes R(t)$, is considered an *ill-posed* convolution
equation because both $F$ and $R(t)$ are unknown ($\frac{k_w}{\rho}$ is an estimated constant and
will be omitted from following equations for simplicity but it is imperative that it is
taken into account during all analyses). There are two approaches for solving Eq. (1.4), either by assuming or not assuming a known function to model \( R(t) \). The model dependent approach generally assumes an exponential or Lorentzian function for \( R(t) \) as a function of MTT (for example \( R(t) = e^{-t/MTT} \)) and uses general nonlinear least squares minimization to calculate \( F \) and MTT (39,40). This approach is usually taken during computer simulations, however in practice a model independent approach is taken due to the uncertainty in assuming a model for \( R(t) \).

The two main methods for model independent deconvolution are transformation and an algebraic approach. Transformation uses the convolution theorem of Fourier i.e. the transform is multiplicative to convolution. Thus, \( F \) and \( R(t) \) can be solved by the following equations (\( \hat{f}\{ \} \) denotes the Fourier transform and its inverse, \( \hat{f}^{-1}\{ \} \)),

\[
\hat{f}\{ C_i(t) \} = F\hat{f}\{ C_a(t) \} \hat{f}\{ R(t) \}
\]

which can be rearranged to show,

\[
FR(t) = \hat{f}^{-1}\left\{ \frac{\hat{f}\{ C_i(t) \}}{\hat{f}\{ C_a(t) \}} \right\}.
\]

This approach is time efficient and easy to implement from a computational standpoint but is severely sensitive to noise. For this reason a Weiner filter and other dampening filters must be used to reduce noise sensitivity (36,41).
The standard approach (algebraic) to calculate CBF begins with rewriting Eq. (1.4) into discretized form,

\[
C_i(t_j) \approx F \Delta t \sum_{i=0}^{j} C_a(t_i) R(t_j - t_i)
\]  

(2.4)

and then into matrices and treating it as an inverse matrix problem,

\[
\begin{pmatrix}
  C_i(t_1) \\
  C_i(t_2) \\
  \vdots \\
  C_i(t_n)
\end{pmatrix} = \Delta t \begin{pmatrix}
  C_a(t_1) & 0 & \cdots & 0 \\
  C_a(t_2) & C_a(t_1) & \cdots & 0 \\
  \vdots & \vdots & \ddots & \vdots \\
  C_a(t_n) & C_a(t_{n-1}) & \cdots & C_a(t_1)
\end{pmatrix} \begin{pmatrix}
  R(t_1) \\
  R(t_2) \\
  \vdots \\
  R(t_n)
\end{pmatrix}
\]  

(2.5)

Eq. (2.5) can be simplified,

\[
c = A \cdot b
\]  

(2.6)

and then solved for \(b\) by inverting \(A\). By singular value decomposition (SVD),

\[
A = U \cdot S \cdot V^T
\]  

(2.7)

where \(U\) and \(V\) are orthogonal matrices (\(^T\) indicates transpose) and \(S\) is a diagonal matrix. Before inverting \(A\), the nonzero values in \(S\) must be filtered by a preset percentage of the maximum value in \(S\), i.e. if an element in \(S\) is less than the product of this percentage and the maximum valued element in \(S\) it is set to zero. Inverting \(A\) yields,

\[
A^{-1} = V \cdot W \cdot U^T, \quad W = 1/S
\]  

(2.8)

and once \(A\) is inverted, Eq. (2.8) can be substituted into Eq. (2.6),

\[
b = V \cdot W \cdot (U^T \cdot c).
\]  

(2.9)
Because $b$ is scaled by $F$, it can be calculated by taking the maximum value of $b$ (40).

To improve the standard SVD approach, advanced algebraic techniques have been investigated such as block-circulant deconvolution (42) and regularization (43,44).

**Sources of Quantification Error**

There are many errors that can influence DSC MRI estimates such as the deconvolution process (42,45-47) and the effects of CA leakage into extracellular-extravascular space on signal (48,49). Another source of error comes from arterial measurements. Even if relatively large arteries such as the middle cerebral artery are sampled, the resolution is sufficiently low that sampled “arterial” voxels are likely to be contaminated by brain parenchyma. The two major issues addressed by the following chapters are outlined below.

The first source of error is modeling the first-pass bolus as an analytical function. This is important because it smoothes raw data and simplifies perfusion quantification (see Eq. (1.12)). The errors concerning the aforementioned along with a practical solution will be discussed in chapter 3.

The second source of error is estimating $C(t)$. It is well known and proven in simple solutions, such as water, relaxivity is linear

$$\Lambda(C) = r_2^* C(t) T_E.$$  \hspace{1cm} (2.10)
However, in heterogeneous material such as tissue, blood and yeast the relationship is decidedly nonlinear (34,35,50-59). The exact magnitude of these errors are unknown, however Calamante et al. suggest that the effects on relative perfusion measurements may be small when compared to absolute (60). The relaxivity in arterial blood has been empirically determined using bulk blood phantoms and follows a quadratic form,

$$\Lambda(C) = (pC + qC^2)T_E$$  \hspace{1cm} (2.11)

where $p$ and $q$ are coefficients which depend on factors such as hematocrit and field strength (53,54). The relaxivity of tissue, venous blood and yeast will be further investigated in chapters 4, 5 and 7, respectively.
CHAPTER 3: The Single Compartment Recirculation Model

The work in this chapter appears in *Medical Physics* under the following citation:


This study was funded in part by NIH grant RO1CA111996.

Author Contributions:

Vishal Patil – Project concept and design, data acquisition and analysis, manuscript drafting and revisions.

Glyn Johnson – Project concept and design, manuscript drafting and revisions, final approval for publication.
Abstract

Quantification of perfusion measurements using dynamic, susceptibility-weighted contrast-enhanced (DSC) MRI depends on estimating the size and shape of the tracer bolus. Typically, the bolus is described as a gamma variate function fit directly to the tracer concentration time curve (CTC), however, there are problems associated with this method. First, the last point to fit is arbitrary and more importantly, direct fitting also includes a portion of the recirculation curve, which in turn overestimates and distorts the true bolus. In this study, we present a model which also describes the bolus as a gamma variate but takes into account recirculation during the bolus and is fit to the entire CTC.
Introduction

When calculating perfusion parameters it is normal to model the concentration time curve (CTC) by an analytic function. Usually this is a gamma variate function fitted to the first pass portion of the CTC

\[ g(t) = A(t-t_0)^\alpha \exp\left(-\frac{(t-t_0)}{\beta}\right); \quad t > t_0 \]  

(3.1)

where \( A \) is a scaling factor, \( \alpha \) and \( \beta \) determine the bolus shape and \( t_0 \) is the bolus arrival time.

An alternative CTC model, that we will call the single compartment recirculation (SCR) has also been proposed (18)

\[ C(t) = g(t) + \kappa \int_{0}^{t} g(\tau)d\tau \]  

(3.2)

where \( \kappa \) is a unitless constant less than one (usually around 0.05 for a DSC MRI CTC). The integral term describes recirculating contrast. This fraction is composed of tracer absorbed as the bolus passes through other tissue compartments and released thereafter and of tracer dispersed through the vasculature through mixing.

We believe that the SCR model is preferable to the alternative gamma variate (GV) model for a number of reasons. First, with the exception of the short time-scale perturbations caused by incomplete dispersion of bolus the SCR model describes the data very well empirically. Second, the gamma variate fit would only be correct if the recirculating fraction of the tracer began only after the first pass of the bolus. In reality, recirculation will contribute some tracer to the circulation during the first pass (61,62). A simple gamma variate fit then overestimates the size
of the bolus. In contrast, the SCR model provides a more realistic account of the contribution of recirculating tracer in the absence of tracer leakage, and there is some theoretical justification for describing recirculating contrast in this way (63). Finally, fitting the SCR model does not involve determining a last point to fit since it fits the entire CTC. This is particularly important since the choice of the last point is somewhat arbitrary and susceptible to noise but can nonetheless cause substantial errors in the fit.

In this study we use computer simulation to investigate the effect on estimates of bolus area and first moment of fitting with the SCR and GV models with different methods of choosing the last fitting point.

Methods

DSC data was acquired from 10 healthy subjects (6 females and 4 males; age range 29-71, age mean and standard deviation 55.8±13.9) at 3 T using a gradient echo EPI sequence. A “noise free” signal time curve was first obtained from each subject by averaging brain signals over a single axial slice inferior to the ventricles. Vascular signals were excluded to eliminate distortions due to the nonlinearities in the relaxivity in pure blood. This was achieved by excluding pixels from the average where the signal drop was over 40% larger than the average signal drop over the entire brain. Fifteen different levels of Gaussian noise giving signal to noise ratios, SNRs, (defined as \( S_{\text{pre}} / \sigma \) where \( \sigma \) is the standard deviation of the pre-bolus signal, \( S_{\text{pre}} \)) from approximately 20 to 130 were applied to this “noise
free” data. This process was repeated 200 times giving a total of 30,000 different simulated signals.

CTCs were modeled by both SCR and GV, converted to signal and fitted to the noisy signal data. Signal, $S$, was calculated using the standard expression. Fitting to the signal is preferable because the weighting for each data point is equal for all signal intensities and because $S_{pre}$ can be included as a fitting parameter, making optimum use of the available data in finding this parameter.

The last fitting point for the GV was determined using four methods (Fig. 3.1): i) third time point after the bolus minimum (64), ii) time point at half the bolus depth after the peak (65), iii) an adaptive method using first time point after the bolus peak within one standard deviation of the post bolus signal, and iv) visual determination (66). Fig. 3.2 shows the noise free CTC (crosses), the bolus (solid line) and recirculation (dotted line) determined by the SCR fit and the bolus (dashed line) determined by a GV fit. The different models were fitted using the alternate form of the gamma variate developed by Madsen (67). This form decouples gamma variate amplitude from the shape parameters and so gives more stable fits,

$$g(t) = y_{max} t^\alpha \exp\left(\alpha(1-t')\right) \quad t' = \frac{(t-t_0)}{(t_{max} - t_0)}$$

(3.3)

where $t_{max}$ is the time when the bolus is at maximum height, $y_{max}$ is the maximum height and $\alpha$ is a decay parameter.
Next, for each fit the area under the curve (AUC) and the normalized first moment (FM) of the bolus (i.e., the gamma variate portion of both SCR and GV models) were calculated using the following equations,

\[
AUC = \int_0^\infty g(t) \, dt = y_{max} \left( \frac{t_{max}}{\alpha} \right)^{\alpha+1} \Gamma(\alpha + 1) \quad (3.4)
\]

\[
FM = \frac{\int_0^\infty tg(t) \, dt}{\int_0^\infty g(t) \, dt} = \frac{t_{max}}{\alpha} (\alpha + 1) + t_0 \quad (3.5)
\]

where \( \Gamma \) is the gamma function. CBV is proportional to AUC and CBF is a function of FM so these two parameters determine the errors that poor fits will introduce into perfusion estimates.

Finally, the percent error (Eq. (3.6)) and percent deviation (Eq. (3.7)) relative to values for the “noiseless” fit, respectively, were calculated

\[
P.E_{SNR=i} = 100 \times \left( \frac{\bar{x} - x_{SNR=\infty}}{x_{SNR=\infty}} \right) \quad (3.6)
\]

\[
P.D_{SNR=i} = 100 \times \sqrt{\frac{1}{N} \sum (x - \bar{x})^2} \quad (3.7)
\]

where \( N \) is the number of trials.
Fig. 3.1 – Diagram of a typical signal time curve illustrating how the last fitted point for the gamma variate was selected for each method: i) third point after the bolus minimum, ii) point of the half drop of the bolus after the bolus minimum, iii) the point after the minimum at which the signal exceeds the post bolus signal minus the standard deviation of the pre bolus signal ($\sigma$) and iv) by visual determination.
Fig. 3.2 – Example of the bolus (solid line) and recirculation (dotted line) portions of the SCR model and bolus (dashed line) of a GV fitted to a typical concentration time curve (crosses).

Results

Fitting proved to be extremely robust for both SCR and GV fits, even at low SNR. All 30,000 fits converged within the maximum allowed value of 200 iterations and most converged in less than 15. We did not observe any fits that appeared to have settled in false minima. The maximum, minimum and median $R^2$ values were 0.99, 0.80 and 0.98 respectively.
Fig. 3.3 shows typical fits for the SCR model (Fig. 3.3a and b) and for each GV method (i-iv) (Fig. 3.3c-j) for the “noiseless” data and with noise added to reduce the SNR to 25.

The percent error and percent deviation of AUC (Fig. 3.4a and b) and FM (Fig. 3.4c and d) for the different models are plotted against SNR in Fig. 3.4. In general GV_{i} (third point) performs poorly for both error and deviation. The SCR model, GV_{ii} (half depth) and GV_{iv} (visual inspection) give similar errors while GV_{iii} (adaptive) performs acceptably for AUC but poorly for FM. The SCR model performs as well as the best GV methods for AUC and rather better for FM.

Mean values of AUC and FM were smaller for the SCR model than values for all gamma variate fitting procedures as one would expect from Fig. 3.2. There were only small differences between mean values for the different GV methods. For example at an SNR of 100, SCR AUC and FM were 7.6 ± 1.73 a.u. and 8.8 ± 2.40 s respectively whereas GV values were 9.4 ± 1.88 a.u. and 10.0 ± 2.51 s.
Fig. 3.3 – Fits (line) to concentration time curves (crosses). The left column gives the “noiseless” concentration time curves and the right has added noise to give an SNR of 25.
Fig. 3.4 – Percent error (a) and percent deviation (b) of the area under the curve (AUC) plotted against SNR for each model. Percent error (c) and percent deviation (d) of the first moment (FM) plotted against SNR for each model.

Discussion

In this study we compared the robustness with respect to noise of fitting DSC data with SCR and GV models with different methods of estimating the last
point. Noise introduces both bias (i.e., systematic errors) and variability into AUC and FM estimates that will propagate to measurements of CBV, CBF and mean transit time (MTT). CBV is proportional to the ratio of the AUCs measured in tissue and artery and it is typical to apply the model functions to both the arterial input function (AIF) and tissue measurements. The variance will be given by

$$\sigma_{CBV}^2 = \left( \frac{\sigma_{A_t}}{A_t} \right)^2 + \left( \frac{\sigma_{A_a}}{A_a} \right)^2$$

(3.8)

where $A_t$ and $A_a$ are the AUCs in tissue and artery respectively and $\sigma_{CBV}$ is the variance in CBV.

The relationship between FM and mean transit time (MTT) is complex and, as far as we are aware cannot be expressed analytically. Overestimates of the MTT of the AIF due to errors will reduce estimated values of tissue MTT and hence increase estimates of CBF. Conversely overestimates of tissue MTT will lead to underestimates in CBF. Errors in CBV will also propagate to CBF. A full analysis is beyond the scope of this chapter.

Our results show that the SCR model is the most robust fitting technique with respect to noise both for bias and variability. The best of the gamma variate methods, GV$_{iv}$, produced similar or slightly worse results to the SCR model; however the last point to fit in this method is determined visually. In this study this was estimated from the “noise free” data since it is impractical to determine it in this way for 30,000 different data sets. However, this is a major practical disadvantage of this method. It is only feasible to apply this method on a pixel by
pixel basis if it is assumed that the length of the bolus is the same for all pixels. Clearly this is not the case and will result in significant errors.

The SCR model corrects for errors that are likely to be smaller than others often associated with DSC MRI. However many of these errors can also be addressed effectively by improved processing and acquisition methods. For example, partial volume effects due to inadequate spatial resolution can be reduced using reference signals acquired from the sagittal sinus (68); signal “clipping” (saturation) can be reduced by reducing tracer dose or echo time; and the nonlinearity between relaxation and concentration can be addressed by using empirically derived calibration curves (53).

There are a number of limitations of the SCR model and of this study. First, the model is based on the assumption that recirculation starts at the beginning of the bolus and increases as a gamma function. Neither of these assumptions can be fully justified. There may be a delay before recirculation contrast arrives and some other function may describe its arrival more accurately. Nonetheless the form of the function must be approximately sigmoidal similar to the form we suggest. Second, we did not consider the possible effects of cardiac output on our results. However, our subjects covered a wide age range (29-71) suggesting that cardiac output has relatively little effect. Third, the SCR model takes no account of errors introduced by incomplete dispersion leading to second or even third pass peaks. These errors might be particularly severe if too few measurements are taken after the bolus so that the steady-state portion of the curve is not reached. We are
currently investigating more sophisticated models that explicitly account for secondary peaks. Conversely, if too many data points are acquired after the bolus, the steady state portion of the curve will start to decline because of tracer clearance through the kidneys. However, this can be accounted for by multiplying the SCR model by an exponential decay term. The model introduces one additional fitting parameter, \( \kappa \), giving the possibility of over-fitting and instability. However, the effect of \( \kappa \) on the shape of the function is so different from those of the other parameters that this is not a serious risk in practice. Finally, the SCR model is subject to many of the same errors as the GV model such as tracer leakage, \( T_i \) contamination and partial volume effects.

In conclusion, we have demonstrated that the SCR model gives a more robust fit in the presence of noise while giving a more realistic representation of tracer boluses than the gamma variate.
CHAPTER 4: $\Delta R_2^*$ Relaxivity in Brain Tissue

The work in this chapter appears in *NMR in Biomedicine* under the following citation:


This study was funded in part by NIH grant RO1CA093992.

Author Contributions:

Vishal Patil – Project concept and design, data acquisition and analysis, manuscript drafting and revisions.

Jens H. Jensen – Project concept and design, manuscript drafting and revisions.

Glyn Johnson – Project concept and design, manuscript drafting and revisions, final approval for publication.
Abstract

Dynamic, susceptibility-weighted, contrast-enhanced (DSC) MRI perfusion measurements depend on estimating intravascular contrast agent (CA) concentration (C) from signal intensity changes in $T_2^*$-weighted images after bolus injection. Generally, linearity is assumed between relaxation and C, but previous studies have shown that compartmentalization of CA and secondary magnetic field perturbations generate deviations from linearity. Physical phantoms using bulk blood have been used to empirically determine the relationship between relaxation rate and C in large vessels. However, the relaxivity of CA in the microvasculature is not easily estimated since constructing appropriate phantoms is difficult. Instead, theoretical relaxivity models have been developed. In this study we empirically tested both linear models of tissue relaxivity and a theoretical model based on the static dephasing regime. Signal-time curves in white matter (WM) and grey matter (GM) were converted to concentration-time curves (CTCs) using a nonlinear expression based on the static dephasing regime (SDR) and a linear approximation. Parameters for both the linear and nonlinear formulations were adjusted to give the best agreement between cerebral blood volumes (CBV) calculated from the WM and arterial CTCs in a group of normal subjects scanned at 3 T. The optimized parameters were used to calculate blood volume in WM and GM in healthy subjects scanned at 3 T and in meningioma patients scanned at 1.5 T. Results from this study show the nonlinear SDR formulation gives an acceptable functional form.
for tissue relaxivity, giving reliable CBV estimates at different field strengths and echo times.
Introduction

If $T_i$ effects are neglected, the signal, $S$, in a DSC MRI experiment is given by

$$S = S_{\text{pre}} \exp\left(-\Lambda(C)\right)$$

(4.1)

where $S_{\text{pre}}$ is the signal before contrast injection and $\Lambda(C)$ is a function describing signal loss due to the presence of CA in the vasculature. (It should be noted $\Lambda(C)$ is a unitless function).

In general, CA concentration corresponding to a particular signal is estimated by first calculating signal log ratios, $\lambda$

$$\lambda = -\ln\left(\frac{S}{S_{\text{pre}}}\right)$$

(4.2)

and applying the inverse of $\Lambda(C)$ either analytically or by means of a lookup table:

$$C = \Lambda^{-1}(\Lambda_0 + \lambda).$$

(4.3)

In most studies a linear relationship between $C$ and relaxation rate is assumed so that

$$\Lambda = rCT_E$$

(4.4)

where $r$ is the CA relaxivity constant. However, studies have demonstrated that this linear relationship, while valid in simple solutions, may fail in more structured media and thus can lead to systematic errors in $C$ quantification in vivo (57,60). Worse, relaxivity is generally dependent on both magnetic field and echo time so that DSC results are protocol dependent and cannot be meaningfully compared.
across studies. For example, the optimal relative CBV (rCBV) cutoff for distinguishing low and high grade gliomas has been reported as anywhere between 1.5 and 5.6 (26-30).

The exact expression for relaxivity depends on the compartmentalization of CA in different tissue types (34,35,51,57,69-72). Studies of gadolinium diethylenetriaminepentaacetic (Gd-DTPA) in bulk blood (and hence large vessels) have found a quadratic relaxivity so that (53,55,71,72)

$$\Lambda = \left( pC + qC^2 \right) T_E$$

(4.5)

where $p$ and $q$ are constants.

However, tissue microvasculature is difficult to replicate in a physical phantom and *in vivo* calibration measurements are difficult to perform. For these reasons, researchers have resorted to theoretical models and Monte Carlo simulations to determine tissue CA relaxivity (34,35,69,73). Two theoretical limiting cases of $T_2^*$ relaxivity have been described: the static dephasing regime (SDR) and the diffusion narrowing regime (DNR). In the latter, the signal dephasing time is long enough for molecular diffusion to average out phase shifts caused by different magnetic moments (74). DNR holds when the diffusion length of a water proton is much greater than the characteristic distance describing the distribution of contrast agent in tissue. Conversely, the SDR holds when diffusion lengths are small.
The SDR, first formulated by Yablonskiy and Haacke (33) for randomly oriented cylinders, is divided into short and long dephasing time regimes:

\[
\Lambda = \begin{cases} 
0.3\varsigma (\omega T_E)^2 & \omega T_E \leq 1.5 \\
\varsigma (\omega T_E - 1) & \omega T_E \geq 1.5 
\end{cases}
\]

(4.6)

where

\[
\omega = \eta \pi \gamma (\chi_0 + kC) B_0
\]

(4.7)

\(\varsigma\) is the tissue vascular fraction, \(\eta\) is a constant that depends on the geometry of the vasculature network, \(\gamma\) is the gyromagnetic ratio, \(B_0\) is the external magnetic field, \(\chi_0\) is the magnetic susceptibility due to deoxygenated red blood cells, and \(k\) is a coefficient relating susceptibility to Gd-DTPA concentration. Yablonskiy and Haacke also give an interpolation formula valid over all \(T_E\) (33):

\[
\Lambda = \varsigma \frac{1}{3} \int_0^1 (2 + u) \sqrt{1 - u} \frac{1 - J_0 \left( \frac{3}{2} \frac{\omega T_E u}{u^2} \right)}{u^2} du
\]

(4.8)

where \(J_0\) is the zeroth order Bessel function and we have included a simplified form for \(\omega\) in the second form of the equation.

A later study by Kjølby et al. using Monte Carlo and suggested that a linear relationship is adequate to describe relaxivity for \(T_E > 10\) ms when using double-dose contrast (73). However, a more recent simulation study (75) suggests a nonlinear relationship is more accurate for single dose.
The purpose of this study was to determine a functional form for $\Lambda$ that allows reliable estimates of $C$ in WM and GM at different field strengths and echo times. To this end, we compared the full (Eq. (4.8)) and linear approximation (the long $T_E$ approximation of Eq. (4.6)) of the SDR model. Constants in these equations can be estimated theoretically (33,73) but depend on factors such as vessel geometry, etc., that are not accurately known. We therefore assumed $\omega = rCB_0$ for the linear model and determined empirically the value of $r$ that gave the best agreement between tissue and arterial bolus curves in control subjects. Similarly, we assumed that $\omega = (a + bC)B_0$ for the full SDR model and found best agreement values of $a$ and $b$. However, during development we observed that the procedure was very insensitive to the $a$ term giving similarly good fits and similar $b$ values (within about 2%) when it was constrained to be zero. This lack of sensitivity can be explained by the shape of the calibration curve (Fig. 4.3). Altering the value of the constant term shifts the relaxivity curve along the concentration axis close to the origin. Because the slope of the curve is close to zero at that point, the change in relaxivity is very small. Our final results were therefore derived with $a$ set to zero. (Note that although this also gives a linear relationship between $C$ and $\omega$, $\Lambda$ is nonlinear with $C$.)

The empirically determined values of $r$ and $b$ were then validated in WM and GM at different field strengths and echo times in a different set of subjects.
Methods

This retrospective study was approved by our Institutional Review Board. Data were obtained from 5 meningioma patients scanned at 1.5 T who had undergone DSC MRI as part of their standard clinical examination and 10 healthy subjects who had been scanned at 3 T. A series of 60 $T_2^*$-weighted single-shot EPI images were acquired from each subject at one second intervals during injection of a standard dose of Gd-DTPA (0.1 mmol/kg) at a rate of 5 ml/sec followed by a 20 ml bolus of saline also at 5 ml/sec. Imaging parameters at 1.5 T were: $T_E = 40$ ms; $T_R = 1000$ ms, field of view, $228 \times 228$ mm$^2$; 7 slices; section thickness, 5 mm; matrix, $128 \times 128$; in-plane voxel size, $1.78 \times 1.78$ mm$^2$; $FA = 90^\circ$ and at 3 T were: $T_R = 1000$ ms, $T_E = 32$ ms, field of view, $230 \times 230$ mm$^2$; 12 slices; section thickness, 3 mm; matrix, $128 \times 128$; in-plane voxel size, $1.79 \times 1.79$ mm$^2$; $FA = 30^\circ$.

All software was written in-house in IDL (ITT Visual Information Solutions, Boulder, CO) and Matlab (Mathworks, Natick, MA). Subject data were divided into two groups: 5 sets at 3 T were used to determine the optimum coefficients in the linear and SDR formulations; 10 test sets were used to test the accuracy of those coefficients.

WM signals were measured in regions of interest (ROIs) of approximately 10 pixels in the frontal lobes. Arterial pixels were automatically selected using the criterion described by Rempp et al. (36). Selected pixels were ordered by fractional
signal drop and the first ten pixels which did not exhibit signal saturation and phase cancellation were averaged.

A basic tenet of DSC MRI is that, in the absence of leakage, the area under the bolus portion of the plasma concentration-time curve (CTC) is equal at all points in the vasculature. The area under the tissue CTC is therefore proportional to the vascular fraction (or blood volume), $\zeta$. Hence, if we know the area, $A_A$, under the bolus estimated in large vessels, we can predict the area under the bolus in white matter, $\hat{A}_{WM}$, using literature values of white matter $\zeta$, and taking into account the different hematocrits found in small and large vessels so that

$$\hat{A}_{WM} = \zeta \frac{1 - H_{SV}}{1 - H_{LV}} A_A$$

(4.9)

where $\zeta$ is the vascular fraction in white matter. In this study we assumed: $\zeta = 0.025$ (76); $H_{LV} = 0.4$ (77); and $H_{SV} = 0.28$ (78).

To find $A_A$, arterial signals were first converted to estimates of log ratio, $\lambda_A$ (Eq. (4.2)) then converted to arterial contrast agent concentration, $C_A$, using the empirically derived quadratic calibration curves, Eq. (4.5), with constants $p$ and $q$ those found in bulk blood with 40\% Hct (53,71): 1.5 T, $p = 7.2 \text{ s}^{-1}\text{mM}^{-1}$, $q = 0.74 \text{ s}^{-1}\text{mM}^{-2}$; 3 T, $p = 0.49 \text{ s}^{-1}\text{mM}^{-1}$, $q = 2.61 \text{ s}^{-1}\text{mM}^{-2}$.

To smooth out noise and simplify calculation of the bolus area, $C_A$, was modeled by an analytic bolus shape function. The most commonly used bolus shape function is the gamma variate function
\[
g(t: y_{\text{max}}, t_{\text{max}}, \alpha) = y_{\text{max}} t^{\alpha} \exp(\alpha (1 - t'))
\]  

(4.10)

where

\[
t' = \frac{(t-t_0)}{(t_{\text{max}}-t_0)}
\]  

(4.11)

\(t_0\) is the start of the bolus, \(t_{\text{max}}\) is the time when the bolus is at maximum height, \(y_{\text{max}}\) is the maximum height and \(\alpha\) is a decay parameter. (This is the modified form of the gamma variate function introduced by Madsen (67) which is more robust for least squares fitting.) However, the gamma variate does not describe recirculation well. We therefore use a model called the single compartment recirculation (SCR) model (79):

\[
C(t) = g(t) + \kappa \int_0^t g(\tau) d\tau
\]  

(4.12)

where \(\kappa\) is a constant less than one (usually about 0.04). This equation is composed of the gamma variate given in Eq. (4.10) and an integral term that describes recirculating contrast. There is some theoretical justification for this model (61) and, empirically, it describes the data well.

The bolus \emph{per se} is represented by the gamma variate portion of Eq. (4.12), so that \(A_t\) is given by

\[
A_t = \int g(t: y_{\text{max}}, t_{\text{max}}, \alpha) dt = y_{\text{max}} \left( \frac{t_{\text{max}}}{\alpha} \right)^{\alpha+1} \Gamma(\alpha+1)
\]  

(4.13)

where \(\Gamma\) is the gamma function.
White matter concentrations were also modeled by the SCR (Eq. (4.12)). In general, \( t_0 \), \( t_{\text{max}} \) and \( \alpha \) will be different in WM and arteries due to delay and dispersion. However, the area under the bolus is set to equal to the predicted value, \( \hat{A}_{\text{WM}} \) (Eq. (4.9)), which constrains the value of \( y_{\text{max}} \). Similarly \( \kappa \) will be equal to the arterial value since the post-bolus concentration is equal in all vessels (35,38). This constrained model for white matter concentration is converted to log ratio values using the linear or nonlinear formulations and fitted to measured values with \( t_0 \), \( t_{\text{max}} \), \( \alpha \) and \( b \) as free parameters.

In summary, the process proceeds as follows:

1. Measure arterial signal and convert to log ratio estimates, \( \dot{\lambda}_A \).
2. Model arterial concentration, \( C_A \), and fit to the SCR model with \( t_0 \), \( t_{\text{max}} \), \( y_{\text{max}} \), \( \alpha \) and \( \kappa \) as free parameters.
3. Calculate the area under the arterial bolus, \( A_A \), and calculate the predicted area under the white matter bolus, \( \hat{A}_{\text{WM}} \).
4. Measure WM signal and convert to log ratio estimates, \( \dot{\lambda}_{\text{WM}} \).
   a. Model WM concentration, \( C_{\text{WM}} \), by the SCR model, convert to log ratio with either the linear or nonlinear formulations and fit to \( \dot{\lambda}_{\text{WM}} \) with \( \kappa \) equal to that found in arteries, \( y_{\text{max}} \) constrained to give bolus area \( A_{\text{WM}}' \) and \( t_0 \), \( t_{\text{max}} \), \( \alpha \), and \( b \) as free parameters.
The derived parameters $r$ (Eq. (4.4)) and $b$ are the coefficients that give the best agreement between estimated and fitted concentration curves assuming linear and nonlinear relaxivities. Uncertainties in $r$ and $b$ were calculated by scaling the formal 1-sigma errors (calculated from the covariance matrix) to the square root of the summed squared residuals divided by the degrees of freedom.

This procedure was carried out on 3 T WM data from the five training subjects. (It should be noted that $B_0$ is equal to 2.89 T on our nominally 3 T systems.) To obtain the best global estimates all fits were conducted simultaneously with different values of SCR parameters for each data set but a single value of $b$ for all sets.

WM and arterial signals were measured as before and GM measurements were taken from the caudate nucleus. The empirically optimized \( \Lambda \) calibration curves were used to calculate estimates of bolus area, \( \hat{A}_{WM} \) and \( \hat{A}_{GM} \) using Eq. (4.9) and the fractional error in relative to literature values was calculated

\[
FE = \frac{A - \hat{A}}{A} \times 100.
\]

Since CBV is proportional to the bolus area under the tissue curve, the \( FE \) in the bolus area is equivalent to the \( FE \) in CBV. Single pixel signal measurements were also made in WM and GM to determine how reproducible CBV measurements in noisy data.

As a further test, WM and GM CTCs were directly compared. WM and GM ROIs are sufficiently close that we would expect little differential delay or
dispersion between the two. The only difference should then be in amplitude. Specifically the GM curve should be larger by a factor $\frac{\varsigma_{GM}}{\varsigma_{WM}} = 3.8/2.5 = 1.52$ (76). The GM CTC values were therefore divided by 1.52 and compared with WM CTCs for both linear and nonlinear formulations.

**Results**

Fig. 4.1 shows a typical $T_2^*$-weighted EPI image at 3 T and the positions of the WM and GM ROIs and pixels. Fig. 2 shows measured (+) and fitted log ratio ($\lambda$) white matter curves in the optimization sets ($B_0 = 3$ T; $T_E = 32$ ms) for both linear (Fig. 4.2a) and nonlinear formulations (Fig. 4.2b). Data from all patients have been concatenated. The linear formulation clearly underestimates the bolus peak and width and overestimates the recirculation portion (tail) of the curves, while the nonlinear formulation yields an excellent fit.

Final optimum coefficients and uncertainty in estimated parameters were $r = 28.9 \pm 0.48$ s$^{-1}$T$^{-1}$mM$^{-1}$ and $b = 114.4 \pm 0.84$ s$^{-1}$T$^{-1}$mM$^{-1}$. Fig. 4.3 shows the two blood calibration curves for WM, $B_0 = 3$ T and $T_E = 32$ ms.

Fig. 4.4 shows calculated blood CTCs derived from arteries (red) and WM in control patients using the linear (blue) and nonlinear (green) formulations in typical test patients at 1.5 T and 3 T (Fig. 4.4a and b), respectively (tissue CTCs were normalized by $\varsigma$ to give blood CTCs). At both field strengths, the arterial curve starts before and is narrower, with higher peak height than the SDR WM
curves, consistent with delay and dispersion between brain and artery. The heights of the arterial and nonlinear curves are very similar in the recirculation phase, after complete bolus dissipation. With the linear formulation, the 1.5 T WM curve appears to somewhat underestimate concentration during both bolus and recirculation phases, at 3 T the linear formulation markedly underestimates concentration during both phase. Similar effects are seen in fits used to determine $b$ (Fig. 4.2a). This suggests that finding a single value of $b$ for the linear formulation that reliably describes relaxivity at different field strengths and echo times is difficult.

Fig. 4.5 gives box and whisker plots of fractional errors of measured CBV (i.e. $A$) from the test data at both 1.5 and 3 T and for both linear and nonlinear formulations taking ROI and single pixel measurements in WM (Fig. 4.5a-b) and GM (Fig. 4.5c-d) and in Table 4.1. With the nonlinear model all values are very close to literature values and the variance is relatively small. The linear model produces a marked bias and large variance in all measurements Results from the individual voxels are noisier than those from the ROIs (as would be expected), but are otherwise similar to the ROI results.

Blood CTCs calculated using linear and nonlinear formulations in WM (blue) and GM (green) from a single test patient are shown at 1.5T (Figs. 4.6a and e) and 3 T (Figs. 4.6c and g), respectively. The remarkable agreement between the two tissue curves at both field strengths suggests that the nonlinear SDR formulation is an excellent functional form for brain tissue relaxivity. Differences
between scaled GM and WM curves from the beginning of the bolus to 30 seconds after are plotted against WM concentration over all test patients at using linear and nonlinear formulations at 1.5 T (Fig. 4.6b and f) and 3 T (Fig. 4.6d and f), respectively. The linear formulation appears to systematically overestimate GM concentration and high concentrations. By contrast, there appears to be no systematic bias to concentration estimates obtained with the nonlinear SDR form.

Fig. 4.1 – $T_2^*$-weighted DSC MRI image acquired at 3 T. Region of interests and single pixels were drawn to measure signal intensities in, WM, normal appearing white matter; and GM, caudate nucleus.
Fig. 4.2 – Plots of measured (+) and fitted values of log ratio, $\lambda$. Each plot is the concatenation of 5 data sets at 3 T, $T_E = 32$ ms. a) Linear formulation. b) Nonlinear formulation.

Fig. 4.3 – Plots of $\Lambda$ vs. $C$ for the optimized linear (dashed) and SDR (solid) formulations ($B_0 = 3$ T; $T_E = 32$ ms).
Fig. 4.4 – Plots of estimated blood contrast agent concentration from a single patient at a) 1.5 T and b) 3 T. The concentration estimates were obtained from signals in arteries (red line), white matter using the linear (blue line) and nonlinear (green line) formulations. The arterial and white matter concentrations calculated using the nonlinear formulation is in excellent agreement when considering delay and dispersion.
Fig. 4.5 – Box and whisker plots of fractional error in ROI and single pixel measurements in white matter (a and b) and grey matter (c and d) CBV estimates obtained using the linear and nonlinear formulations at 1.5 T and 3 T. In each box, the central line represents the median of measurements in five test patients, the upper and lower boundaries of the box represent the upper and lower quartiles and the whiskers represents the range.
Table 4.1 – Fractional error in white and grey matter CBV estimates (%) relative to literature values by the linear and nonlinear equations at 1.5 and 3T for both ROI and single voxel measurements.

<table>
<thead>
<tr>
<th></th>
<th>1.5 T</th>
<th>3 T</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ROI</td>
<td>Voxel</td>
<td>ROI</td>
<td>Voxel</td>
</tr>
<tr>
<td>WM</td>
<td>Linear</td>
<td>-10.1% ± 18.8</td>
<td>17.2% ± 17.7</td>
<td>46.2% ± 9.4</td>
</tr>
<tr>
<td></td>
<td>Nonlinear</td>
<td>0.5% ± 3.1</td>
<td>6.5% ± 9.1</td>
<td>-0.3% ± 1.8</td>
</tr>
<tr>
<td>GM</td>
<td>Linear</td>
<td>40.7% ± 18.6</td>
<td>57.0% ± 23.6</td>
<td>123.9% ± 19.1</td>
</tr>
<tr>
<td></td>
<td>Nonlinear</td>
<td>-1.1% ± 5.1</td>
<td>-1.6% ± 11.4</td>
<td>0.7% ± 1.8</td>
</tr>
</tbody>
</table>
Fig. 4.6 – Blood concentration-time curves in WM (blue) and GM (green) from a single test patient calculated using linear and non-linear formulations at 1.5 T (Figs.
4.6a and e) and 3 T (Figs. 4.6c and g), respectively. Differences between scaled GM and WM curves from the beginning of the bolus to 30 seconds after are plotted against WM concentration over all test patients at using linear and non-linear formulations at 1.5 T (Fig. 4.6b and f) and 3 T (Fig. 4.6d and f), respectively.

Discussion

Estimating contrast agent concentration from changes in relaxation rate is the essential first step in making contrast-based MRI perfusion measurements. Without an accurate relationship, absolute quantification of perfusion parameters is impossible. Furthermore, because relaxivity in tissues may be a complex function of contrast agent concentration, field strength and echo time (i.e., it is not necessarily a simple linear function), an accurate comparison of results between sites using different imaging protocols is challenging. Although the theory of relaxivity is quite well established it consists of a number of different regimes and it is sometimes unclear which is applicable in practice. We believe that this study represents the first attempt to compare different functional forms for relaxivity – a linear approximation and a nonlinear interpolation formula – empirically.

It is clear from Figs. 4.2 and 4.3 that the linear approximation underestimates high values of concentration and overestimates low values. Difficulty in reconciling the over- and underestimates with the measured data will contribute to the errors in accuracy seen in Fig. 4.5, and it seems likely may also contribute to the poor precision. All patients underwent the same injection protocol,
if a different injection rate were used or if patients had variable cardiac output, we would expect to see greater errors. Finally, the apparently systematic difference in errors at 1.5 and 3 T suggest that a single linear relaxivity constant cannot adequately describe relaxivity at different field strengths or echo times.

The results using the linear function suggest that the correct function should have an increasing slope. That is relaxation rate must change relatively slowly with concentration at low concentration and faster at high concentration. The nonlinear formulation is therefore of the more appropriate form (Fig. 4.3) and gives excellent agreement between: 1) experimental and fitted curves (Fig. 4.2); 2) arterial and WM derived blood CTCs (Fig. 4.4) after considering dispersion; 3) CBV values for WM and GM (Fig. 4.5); 4) WM and GM CTCs (Fig. 4.6). The agreement appears equally good at the two field strengths and echo times suggesting that the formulation should hold good for a variety of different imaging protocols.

The very low values of fractional error seen with the nonlinear model in Fig. 4.5 are partially because the calibration method used here forces measured values towards the assumed, literature value. (I.e., fractional error is relative to the assumed value not to the true value which is unknown.) However, calibration coefficients were derived from white matter measurements and then applied to grey matter in test subjects. Agreement with literature values of grey matter (which had not been used in the calibration) were nonetheless excellent. Furthermore, the same calibration procedure was used to obtain optimum linear coefficients which still produced an apparent bias in estimates, once again confirming that a linear model is...
inadequate for describing relaxivity. The systematic overestimation of CBV at 3 T relative to 1.5 T emphasizes the problems in finding a single linear relaxation relationship that fits multiple field strengths. The bias in the linear results is due to the inability of linear expression to adequately track the bolus portion of the curve (Fig. 4.4).

Calibration values were calculated on the basis of PET literature values of WM blood volume, the gold standard. The low fractional error in WM test estimates relative to the same literature values seen in Fig. 4.5 might therefore be expected. However, GM literature values were not used in the calibration procedure, only in testing, and therefore cannot explain the low fractional error in these estimates. Furthermore, the same literature values of WM were used to obtain the linear calibration curves which nonetheless demonstrate large biases. Also, the low variance of these estimates at both field strengths suggests that even if not accurate they should at least be consistent across field strengths and echo times. Note also, that although it is not often made explicit, the use of literature values of brain blood volumes is universal. Since the relaxivity of contrast in brain microvasculature is unknown, but much greater than that in arteries, it is necessary to introduce a calibration factor to obtain realistic estimates of CBV.

The values for the coefficient $b$ can be found theoretically using Eq. (4.7):

$$\gamma = 2.675 \times 10^8 \text{ rad} \cdot \text{s}^{-1} \cdot \text{T}^{-1}, \quad k = 0.027 \text{ ppm} \cdot \text{mM}^{-1} \quad (80), \quad b \text{ is 30 for isotropically distributed vessels (} \eta = \frac{4}{3} \pi \quad (33) \text{) and 45 for vessels perpendicular to the}$$
external magnetic field \( (\eta = 2\pi \ (81)) \). This compares quite well with the optimum value of 28.9 found for the linear formulation, but is very far from the optimum of 114.4 found for the nonlinear formulation. There are number of possible explanations for this. First, Hct and oxygen extraction fraction (OEF) levels vary depending on age, gender and medical conditions and treatment. However, although a rather large effect is seen in the empirical studies of whole blood (53,55,82), it is much reduced when plasma concentration is considered. That is, the relaxivity of X moles of CA per ml of plasma is very similar regardless of Hct or OEF. Since our calibration procedure (Eq. (4.9)) takes Hct explicitly into account, the effect on our results will be small. Second, the SDR formula is based on modeling the vasculature as infinitely long straight cylinders, which is an idealization that may introduce errors into theoretical estimates. However, it again seems unlikely that these errors will be sufficient to explain the discrepancy. The most likely explanation may lie in inaccuracies in the interpolation formula, Eq. (4.8). Although the formula is reliable at low and high concentrations, and is of a reasonable functional form, its accuracy is unknown at intermediate concentrations. The slope of the linear asymptote of Eq. (4.6) may critically affect the curvature of the interpolation section. Since the brain parenchyma concentrations on which our calibration is based fall into this intermediate range, our results will be biased towards finding good agreement in this range at the expense of accuracy at high concentrations. Support for the hypothesis that the form of the interpolation determines the empirical optimum of \( b \) is given by our finding that the alternative
interpolation formula of Kiselev (35) gives a very different optimum (~220, even farther from the theoretical value). Better agreement may therefore be found with alternative interpolation formulae. Nonetheless, the empirically optimum value of $b$ used with Eq. (4.8) gives excellent agreement over the range of concentrations found in normal brain with standard doses of contrast. Furthermore, the success of our calibration values in predicting grey matter CBV suggests applicability when vascular density is moderately increased. However, these values should be used with caution at very high values of $C$ where the linear range applies. Moreover, in areas where vessel density is greatly increased, the effects of diffusional narrowing may begin to invalidate Eq. (4.6). Thus, oncological applications of this methodology to highly vascularized neoplasms should be considered carefully and quantitatively.

The close agreement between grey and white matter measurements seen in Fig. 4.6 suggests that the static dephasing regime is adequate to describe relaxivity in normal tissue and therefore that diffusional narrowing (83) is negligible in these circumstances.

There are a number of limitations in this study. 1) We did not attempt to measure Hct or OEF. 2) The empirically determined bulk blood relaxivity curves used in this study were created using a different Gd chelating agent, Optimark gadoversetamide (Gd-DTPA-BMEA), than that used in our study. However calibration curves obtained at 1.5 T using different compounds show only small differences. 3) We have considered only Gd contrast agents. Different calibration
values are likely to be found with other paramagnetic agents or superparamagnetic agents (e.g., ultra-small, superparamagnetic iron oxide particles). 4) Our control subjects at 1.5 T were meningioma patients. Although it would have been preferable to use healthy subjects, it proved difficult to recruit subjects given concerns over nephrogenic systemic fibrosis. However, meningiomas are extra-axial tumors that do not infiltrate into normal brain, and there is no reason to suppose they substantially alter the vasculature. This is confirmed by our finding that CBV estimates in these patients agree with values obtained in controls at 3 T.

5) It would have been preferable to obtain our calibration values of WM blood volume by direct measurements of our subjects using the gold standard of PET. We cannot therefore claim that our results in Fig. 4.5 are accurate but merely consistent with expected values. 6) Finally, this calibration was based on estimates of the arterial input function. This is notoriously difficult to estimate due to partial volume effects. To some extent the procedure presented here is self-correcting in that the coefficients are chosen to give the expected, literature values. That is, if the area under the AIF were reduced by a factor $\alpha$, then the empirical coefficients $r$ and $b$ would be increased by a similar factor to generate the expected CBV value. (Note, however, that in the nonlinear case this would also produce distortions in the CTC. That none are seen in Figs. 4.4 and 4.6 provides evidence that AIF partial volume effects were small in this study.) Provided partial volume effects in our subjects were typical, it should not affect the consistency across protocols (rather than precision) of results obtained with these calibration values. Furthermore, the small
variance in measured results suggests that AIFs were, in practice, relatively reproducible from patient to patient. This may be because we took particular care to exclude pixels where “spiky” boluses or saturation suggested phase cancellation due to partial volume effects.

In conclusion, we have derived a calibration curve that gives consistent estimates of contrast agent concentration from measured relaxivity changes in white matter and grey matter at different values of $T_E$ and $B_0$ and which should be applicable in a wide range of conditions. The function has the same functional form as the nonlinear interpolation formula of Yablonskiy and Haacke (Eq. (4.8)) with $\omega = 114.4 CB_0$. Linear approximations are not adequate to describe relaxivity in brain parenchyma. To our knowledge, this study represents the first attempt to confirm any theoretical form of $T_2^*$ relaxivity in tissue experimentally and the first to provide a reliable expression for relaxivity in the brain.
CHAPTER 5: $\Delta R_2^*$ Relaxivity in Venous Blood

The work in this chapter appears in *Magnetic Resonance in Medicine* under the following citation:


This study was funded in part by NIH grant RO1CA111996.

Author Contributions:

Vishal Patil – Project concept and design, data acquisition and analysis, manuscript drafting and revisions.

Glyn Johnson – Project concept and design, manuscript drafting and revisions, final approval for publication.
Abstract

The accuracy of perfusion measurements using dynamic susceptibility contrast (DSC) MRI depends on estimating contrast agent concentration in an artery, i.e., the arterial input function (AIF). One of the difficulties associated with obtaining an AIF are partial volume effects (PVEs) when both blood and brain parenchyma occupy the same pixel. Previous studies have attempted to correct AIFs which suffer from PVEs using contrast concentration in venous blood. However, the relationship between relaxation and concentration ($C$) in venous blood has not been determined in vivo. In this chapter a previously employed fitting approach is used to determine venous relaxivity in vivo. In vivo relaxivity is compared to venous relaxivity measured in vitro in bulk blood. The results show that the fitting approach produces relaxivity calibration curves which give excellent agreement with arterial measurements.
Introduction

Measurements of venous concentration can be used to automate arterial pixel selection and correct for partial volume effects in the arterial input function (AIF, i.e., the concentration of tracer in arteries) (84-86). These methods have assumed that the relaxivity of arterial and venous blood are the same. However, this is not generally the case as the presence of paramagnetic deoxyhemoglobin in will contribute to venous relaxivity. A recent study by Blockley et al. empirically determined the relaxivity of venous blood in vitro at different field strengths using a blood phantom doped with a Gd based tracer (56). This study found that relaxivity was quadratic with the minimum at some positive value of Gd concentration, $C$. I.e., that relaxivity initially decreases with increasing $C$, as the presence of paramagnetic Gd decreases field differences between diamagnetic plasma and paramagnetic deoxygenated red blood cells. This would result in an increase in signal intensity at the beginning of the bolus but this behavior does not appear to be observable in vivo.

The aim of this note is therefore to determine the relaxivity of venous blood by a fitting approach previously used in Ch. 4 to determine brain parenchyma relaxivity (87). The approach determines the quadratic venous relaxivity function that gives the best agreement between the areas under arterial and venous concentration-time curves (CTCs).
Methods

The study was approved by the Institutional Review Board of this institution. DSC data was acquired from 6 meningioma subjects at 1.5 T ($T_R = 1$ s; $T_E = 47$ ms; $FA = 40^\circ$; matrix $128 \times 128$; FOV $228 \times 228$ mm$^2$; $7 \times 5$ mm slices) and 3 T ($T_R = 1$ s; $T_E = 32$ ms; $FA = 30^\circ$; matrix $128 \times 128$; FOV $230 \times 230$ mm$^2$; $10 \times 5$ mm slices) using a gradient echo EPI sequence during injection of 0.1 mmol/kg Gd-DTPA.

Arterial and venous pixels are detected semi-automatically using adaptations of techniques first suggested by Rempp et al. (36). First, signal was averaged over all images at each time point to obtain an average brain signal. Although it might be preferable to extract the brain first, in practice this average is dominated by brain pixels. The following parameters are then measured: fractional signal drop (FSD), full width at half drop (FWHD) and arrival time (AT, defined as the time at which signal is decreased by 10% of the maximum FSD). Candidate arterial pixels were defined as those with a FWHD at least 1 s less than the average brain value and an AT at least 1 s before the average brain value. Similarly candidate venous pixels were defined as those 1s wider and 1s later than the average. Candidate pixels were visually inspected and the ten with the greatest FSD that showed no evidence of signal saturation, “spiky” boluses, indicative of phase cancellation with surrounding parenchymal pixels (54) were averaged to produce arterial and venous measurements for each patient.
Signal intensity in a $T_2^*$-weighted EPI acquisition (neglecting $T_1$ effects) is given by

$$S = S_{\text{pre}} \exp \left( \Lambda(0) - \Lambda(C) \right)$$

(5.1)

where $S_{\text{pre}}$ is the pre-bolus signal and $\Lambda$ is a function of concentration. The relaxivity of arterial bulk blood has been measured empirically at multiple field strengths and has been found to follow a quadratic form:

$$\Lambda(C) = \left( qC^2 + pC \right)T_E.$$  

(5.2)

At 1.5 T, $q = 0.74 \text{ s}^{-1}\text{mM}^{-2}$ and $p = 7.62 \text{ s}^{-1}\text{mM}^{-1}$ (71); at 3 T, $q = 2.61 \text{ s}^{-1}\text{mM}^{-2}$ and $p = 0.5 \text{ s}^{-1}\text{mM}^{-1}$ (53). (Hematocrit was 40% in both cases.)

The *in vitro* relaxivity of venous bulk blood with a 30% oxygen extraction fraction (OEF) follows a parabola but with a shift ($a$) along the concentration axis

$$\Lambda(C) = \left( q(C-a)^2 + pC \right)T_E.$$  

(5.3)

At 1.5T, $q = 6 \text{ s}^{-1}\text{mM}^{-2}$, $p = -11 \text{ s}^{-1}\text{mM}^{-1}$ and $a = -0.046 \text{ mM}$; 3T, $q = 18 \text{ s}^{-1}\text{mM}^{-2}$, $p = -35 \text{ s}^{-1}\text{mM}^{-1}$ and $a = 0.037 \text{ mM}$ (hematocrit 42%) (56).

In this study we assumed the same form for relaxivity as found in bulk blood, Eq. [3], but found the parameters $q$, $p$ and $a$ that gave the best agreement between arterial and venous CTCs by the following fitting procedure similar to that used previously used to determine parenchymal relaxivity (87).
All CTCs were fitted to the SCR model (Eq. (3.2) (79). The SCR model is preferable to the alternative of fitting a gamma variate to the bolus portion of the CTC alone for a number of reasons.

The procedure is based on the basic postulates of DSC MRI that 1) the area under the bolus (Eq. (3.4)), $A$, must be equal for arterial and venous (and all other vascular) CTCs and 2) the height of the recirculation portion of the CTCs must also be equal. The values of $q$, $p$ and $a$ that best realize these conditions were found by simultaneously fitting CTCs from 5 patients at both 1.5 and 3 T. Briefly, for each patient, $A$ and $\kappa$ are estimated in the artery by modeling the CTC as Eq. (3.2), converting to signal using Eq. (5.1) and the bulk arterial blood relaxivity curves (Eq. (5.2)) and fitting to the measured signal with $S_{pre}$, $y_{max}$, $t_0$, $t_{max}$, $\alpha$ and $\kappa$ as free parameters. Similarly each vascular CTC is modeled by Eq. (3.2), converted to signal using Eqs. (5.1) and (5.3) and fitted to the measured signal. However, the venous fit is constrained to give equal values of $A$ and $\kappa$ to those found in the artery but with the relaxivity parameters $q$, $p$ and $a$ as fitting parameters in addition to $S_{pre}$, $t_0$, $t_{max}$ and $\alpha$. Fits to all 5 patients were performed simultaneously with different gamma variate parameters for each patient but the same relaxivity parameters for all. Since we are comparing two pools of blood with the same hematocrit, it is not necessary to correct for this. Fitting to signal rather than estimates of concentration avoids errors associated with the nonlinear
relationship between noise and concentration (55,88). Including $S_{pre}$ as a fitting parameter is somewhat unusual but should provide the best overall fit of the data.

Results

Fig. 5.1 shows measured (points) and fitted (line) venous signal measurements for 5 patients at 1.5 (a) and 3 T (b). Table 5.1 gives the coefficients for venous relaxivity (Eq. (5.3)) derived from in vivo fitting. It should be noted the $a$ term is very small and can in practice be neglected. This finding is in contrast to that in in vitro bulk blood (56) measurements. Fig. 5.2 shows relaxivity calibration curves for arterial (red) blood, in vitro bulk venous blood (56) (black; coefficients are given in the methods section) and in vivo venous blood (blue) at 1.5 (a) and 3 T (b).

Fig. 5.3 shows CTCs derived for arterial signals (red), and venous signals using in vitro bulk blood relaxivity (black) and in vivo venous relaxivity expressions (blue) at 1.5 (a) and 3 T (b). (The discontinuities in the in vitro curves are due to the ambiguity in relaxivity estimation caused by the non-mononotic nature of the in vitro relaxivity curve. Values between zero concentration and the first non-ambiguous concentration have therefore been derived by interpolation.) With the in vivo relaxivity, the relative widths, heights and arrival times of the arterial and venous curves are consistent with what would be expected from delay and dispersion: the arterial bolus starts before and is narrower and higher than the venous, the recirculation portion of the curve is of similar height. The venous CTC
derived by the in vitro calibration curves are in very poor agreement with what would be expected from the arterial curves. The recirculation portion of the curve is grossly overestimated at both fields; at 1.5 T the bolus is both higher peak and broader than the arterial curve which is not physiologically feasible.

Fig. 5.1 – Measured (points) and fitted (solid lines) venous signal intensity time curves from five patients at 1.5T (a) and 3 T (b). The fitted curves were calculated using the relaxivity parameters that gave the best agreement between the area under the bolus, and steady state amplitude of arterial and venous concentration time curves.
Table 5.1 – Relaxivity coefficients for Eq. (5.3) (curves shown in Fig. 5.2).

<table>
<thead>
<tr>
<th></th>
<th>q</th>
<th>p</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial</strong></td>
<td>1.5 T</td>
<td>0.74</td>
<td>7.62</td>
</tr>
<tr>
<td></td>
<td>3 T</td>
<td>2.61</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>In vivo fitting</strong></td>
<td>1.5 T</td>
<td>0.0018</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>3 T</td>
<td>0.74</td>
<td>12.1</td>
</tr>
<tr>
<td><strong>In vitro bulk blood</strong></td>
<td>1.5 T</td>
<td>6</td>
<td>-11</td>
</tr>
<tr>
<td></td>
<td>3 T</td>
<td>18</td>
<td>-35</td>
</tr>
</tbody>
</table>

Fig. 5.2 – Arterial (red) and venous (in vitro – black; in vivo – blue) relaxivity calibration curves at 1.5 T (a) and 3 T (b). Curve coefficients are given in Table 5.1.
The discontinuities in the in vitro venous curves are because the same value of $\Delta R_2^*$ corresponds to two different concentrations below about 2 mM (see Fig. 5.2). The curve was therefore obtained by interpolation between zero and the first unambiguous value of $\Delta R_2^*$.

Discussion

Theoretical modeling of Gd relaxivity in blood is a difficult problem and, as far as we are aware, has not yet proved amenable to analysis. Relaxivity expressions have therefore been found empirically using phantoms and been shown to resemble a quadratic for both arterial and venous blood. In this study we therefore empirically determined the quadratic function that best describes relaxivity in in vivo venous blood at 1.5 and 3 T. The excellent fits in Fig. 5.1 confirm that quadratic functions adequately describe venous relaxivity although
they do not prove that this is the true functional form. The agreement between arterial and venous CTCs derived from the \textit{in vivo} relaxivity expressions (Fig. 5.3) further confirm this.

The quadratic term in the 1.5 T \textit{in vivo} relaxivity expression is very small, unlike that in other measurements. Again, theoretical modeling is difficult and we have no good explanation for this finding. However, we do note that similar findings are seen in arterial measurements.

The \textit{in vitro} relaxivity curves (Fig. 5.2) give negative $\Delta R_2^*$ over a critical range of concentrations that include those seen during recirculation (between 0.5 and 1 mM – Fig. 5.3). If the arterially derived concentrations are correct the \textit{in vitro} curves would predict a distinct signal increase at the beginning of the bolus and a recirculation portion greater than $S_{pre}$. Neither of these are seen in any venous measurements (Fig. 5.1).

Relaxivity is largely determined by magnetic field inhomogeneities caused by susceptibility differences between diamagnetic plasma, paramagnetic deoxyhemoglobin confined to the red blood cells (RBCs), paramagnetic contrast agent within the plasma and the diamagnetic surroundings (tissue \textit{in vivo}, air or water \textit{in vitro}). In large phantoms, inhomogeneities will be dominated by differences between plasma and RBCs. As the concentration of contrast agent increases these inhomogeneities will initially decrease until the susceptibility of the plasma equals that of the RBCs, creating a minimum in the relaxivity curve shifted to the right along the concentration axis. In small vessels, however, susceptibility
differences between the lumen and surrounding tissue will also contribute to inhomogeneity and hence relaxivity. The initial dose of contrast agent will increase these inhomogeneities thus potentially negating the effect of RBC/plasma differences. This could explain the discrepancy between \textit{in vitro} and \textit{in vivo} relaxivities. If this explanation is correct it suggests that the relaxivity parameters derived here will be somewhat dependent on vascular diameter and should therefore be used with caution in veins different in size from cerebral veins. (Note that this effect would have less effect on \textit{in vitro} measurements of arterial blood since oxygenated RBCs are diamagnetic.)

There a number of limitations to this study: First, DSC data must be acquired with sufficient temporal resolution to capture the passage of the bolus so relatively low spatial resolution acquisitions are employed. Blood vessels are therefore likely to be smaller than the dimensions of pixels so that vascular measurements are contaminated by partial volume effects (PVEs). This study is partially resistant to errors from simple PVEs where the bolus is reduced in amplitude without distortion since the calibration procedure uses the \textit{relative} size of different portions of the CTC (by constraining $\kappa$) (38). However, PVEs can also result in phase cancellation of signal between vessels and surrounding tissue (54,89). Such phase errors can introduce distortions in bolus shape and errors into the calibration. These errors also usually cause characteristically “spiky” and square signal drops that approach zero. For this reason we visually inspected the candidate arterial and venous curves and rejected those showing these effects.
Although this procedure is not certain, the consistent fits achieved (Fig. 5.1) suggest that any residual effects are relatively small. Second, hematocrit and OEF will influence relaxivity (55) so that large variations in these parameters will reduce the accuracy of our calibration values. However, a previous study by Akbudak et al. demonstrates that provided plasma concentrations are compared, hematocrits between 30% and 40% do not produce large differences in $R_i^2$ within the range of concentration in which we are interested (0-5 mM) (53). Thirdly, the in vitro experiments used ProHance as a gadolinium chelate, not Gd-DTPA. This may explain some of the discrepancies though there appears to be little evidence in the literature to suggest major relaxivity differences between most contrast agents. Lastly, the in vitro relaxivity curve was determined over a smaller range of concentrations than the in vivo so that the latter may be more reliable at high concentrations.
CHAPTER 6: Perfusion in Normal Appearing White Matter in Glioma Patients Treated with Bevacizumab: A Clinical Study

The work in this chapter is undergoing review in a peer-reviewed journal and appears in Proceedings of ISMRM 20th Annual Meeting, Melbourne, Australia under the following citation:


This study was funded in part by NIH grant RO1CA093992.

Author Contributions:

Vishal Patil – Project concept and design, data acquisition and analysis.

Manu Singh – Data analysis.

Maya Mathew – Data acquisition.

Ashwathan Narayana – Project concept and design.

James Babb – Data analysis.

David Zagzag – Project concept and design.

Glyn Johnson – Project concept and design, manuscript drafting and revisions.
Abstract

We previously made the incidental observation that MRI estimates of cerebral blood volume (CBV) were reduced in the contralateral white matter (WM) of glioma patients treated with bevacizumab. The purpose of the study was therefore to investigate changes in MRI perfusion measurements that accompany treatment with bevacizumab. Dynamic, susceptibility-weighted, contrast-enhanced (DSC) MRI was performed on three groups of glioma patients: Group I. Newly diagnosed patients treated without bevacizumab. Group II. Newly diagnosed patients receiving bevacizumab. Group III. Patients receiving bevacizumab on recurrence. Serial post-treatment scans were performed in all groups. Serial pre-treatment scans were available for Group III. Estimates of CBV and cerebral blood flow (CBF) were obtained in contralateral white and grey matter (GM) from all scans. Statistically significant reductions in CBV were seen in WM and GM in Groups II (p < 0.01) and III (p < 0.0001). CBF was reduced in WM in Group II and WM and GM in groups II and III (p < 0.05). No significant changes were seen in group I. Post-treatment rates of change CBV were negative in WM and GM (p < 0.01) in Groups II and III. No significant rate of change of CBV was found in group I or in the pre-treatment measurements of Group III. No significant rate of change was found in CBF in either tissue in any group. Reductions in CBV and CBF are seen in normal appearing contralateral brain tissue in glioma patients treated with bevacizumab. CBV reductions increase with time even after treatment has ended.
Introduction

Bevacizumab (Avastin®, Genentech, San Francisco, CA) was the first FDA approved anti-angiogenic agent and has shown efficacy in a number of different tumors including glioma when used in combination with conventional cytotoxic agents (90,91). We have recently been investigating the use of perfusion measurements obtained by dynamic, susceptibility-weighted, contrast-enhanced (DSC) MRI in assessing response in glioma patients treated with bevacizumab. During the course of this investigation we made the incidental observation that blood volumes in normal appearing white matter (NAWM) appeared reduced in these patients. In this study we therefore investigated whether this observation could be confirmed and whether it was associated specifically with bevacizumab treatment.

Methods

This retrospective study was approved by the Institutional Review Board of this institution. DSC MRI scans 1.5 T were: \( B_0 = 1.5 \, \text{T} \); \( T_E = 40-47 \, \text{ms} \), \( T_R = 1000 \, \text{ms} \); field of view, 228×228 mm\(^2\); 10 slices; section thickness, 5 mm; matrix, 128×128; in-plane voxel size, 1.78×1.78 mm\(^2\); \( FA = 40-90^\circ \) were acquired using a gradient echo EPI sequence during injection of 0.1 mmol/kg Gd-DTPA from three groups of patients: Group I received a combination of radiotherapy and chemotherapy but no bevacizumab at initial diagnosis. Group II received bevacizumab and temozolomide after surgery and radiotherapy following initial
diagnosis. Group III received bevacizumab and irinotecan after tumor recurrence, having previously received radio- and chemotherapy without bevacizumab at initial diagnosis. Most patients underwent resection at initial diagnosis. Newly diagnosed patients were scanned immediately before treatment and at 2-3 month intervals thereafter. Recurrent glioma patients were scanned at 2-3 month intervals between initial diagnosis and recurrence and at similar intervals subsequently.

Absolute cerebral blood volume (CBV), cerebral blood flow (CBF) and mean transit time (MTT) were calculated using standard methods that will be summarized here. Tracer concentration is first calculated from the measured signal change using empirically derived calibration curves (59). Total tissue concentration of tracer, $C_t$, is given by the expression:

$$C_t(t) = CBF \cdot R(t) \otimes C_a(t)$$

(6.1)

where $R(t)$ is the tissue residue function, i.e., the fraction of tracer arriving in the tissue at time zero that remains at time $t$, $C_a$ is the concentration in the artery feeding the tissue $\otimes$ represents convolution and we have neglected terms related to tissue density and hematocrit. Since $R(0)$ equals one by definition, estimates of CBF can be obtained by deconvolving $C_t$ by $C_a$. This was achieved using block-circulant singular value decomposition (40,42) with Tikonov regularization (44). Other parameters are calculated from the following expressions (32):

$$MTT = \int_0^\infty R(t)dt$$

(6.2)
and

\[ CBV = CBF \cdot MTT \]  \hspace{1cm} (6.3)

\( C_a \) was measured using a semiautomatic approach adapted from techniques first suggested by Rempp et al. (36).

Signal time curves were measured in normal appearing grey and white matter in the caudate nucleus and frontal lobes contralateral to the tumor. CBV, CBF and MTT were calculated for these ROIs as described above. Measurements were made both before and after treatment. Most patients had multiple post-treatment measurements between 2 and 120 months after treatment. Most recurrent glioma patients (Group III) had multiple measurements both before and after treatment.

The change in each perfusion parameter between the last pre-treatment and first post-treatment scan was assessed using a paired sample t test. Where multiple pre- (Group III) or post-treatment measurements (all groups) were available the rate of change (i.e., slope vs. time) of each parameter was assessed by mixed model regression. The analysis was stratified by measure, tissue type (grey or white matter) and epoch (before vs. after treatment). In each case, the perfusion measures at all pertinent available time points constituted the dependent variable and the model included elapsed time from baseline (in months) as a fixed numeric factor and subject ID as a random classification factor (to account for inter-subject differences in mean perfusion levels and to account for the correlation among
measures from the same subject). Statistical tests were conducted at the two-sided 5% comparison-wise significance level using SAS 9.3 (SAS Institute, Cary, NC).

**Results**

Table 6.1 summarizes the demographics and histological diagnoses of the different patient groups.

Fig. 6.1 and Table 6.2 the value of each perfusion measure at the last time point before treatment and the first time point after treatment for each group. No statistically significant changes were seen in any perfusion parameter in the group that did not receive bevacizumab (Group I). CBF fell by between 19% and 25% in all tissues in the two groups that did receive bevacizumab (Groups II and III). All changes but that in GM in Group II were statistically significant (p = 0.04 or less; values are given in Table 6.2). CBV fell by between 9% and 15% in all tissues in both groups treated with bevacizumab (p = 0.002 or less). All changes were statistically significant. No statistically significant changes were seen in MTT values.

Fig. 6.2 and Table 6.3 give the rates of change of each perfusion measure before and after treatment. No rate of change of any parameter in any pre-treatment groups was significantly different from zero i.e., all pre-treatment parameters were constant to within the measurement precision. However, in both groups treated with bevacizumab (Groups II and III) CBV fell significantly in both GM and WM in the
months after treatment. No other statistically significant rates of change were observed.

Table 6.1 – Patient demographics for each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>M/F</th>
<th>Age Median (range)</th>
<th>Grade (II/III/IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>7/1</td>
<td>59 (41-77)</td>
<td>0/5/3</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>8/2</td>
<td>56 (33-75)</td>
<td>0/3/10</td>
</tr>
<tr>
<td>III</td>
<td>24</td>
<td>15/9</td>
<td>42 (12-78)</td>
<td>2/8/14</td>
</tr>
</tbody>
</table>
Fig. 6.1 – Perfusion parameters for the different subject groups measured pre- (deep gray) and post-treatment (light grey). White and grey matter measurements are given in the top and bottom rows respectively. Statistically significant differences are indicated by asterisks (p values can be found in Table 6.2).

Table 6.2 – The values of each perfusion measure (Mean ± SD) at the last time point pre-treatment and the first time point post-treatment; the change in value and the p value from a paired sample t test. Units are as follows: CBF, ml/100 g/min; CBV, ml/100g; MTT, s. Statistically significant changes are shown in bold.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue</th>
<th>Measure</th>
<th>Pre-</th>
<th>Post-</th>
<th>Change / %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I WM</td>
<td>CBF</td>
<td>29.7 ± 7.0</td>
<td>25.2 ± 5.9</td>
<td>-15</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBV</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MTT</td>
<td>5.5 ± 1.3</td>
<td>6.6 ± 1.4</td>
<td>19</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBF</td>
<td>51.1 ± 11.5</td>
<td>61.1 ± 19.5</td>
<td>20</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBV</td>
<td>4.0 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>-5</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MTT</td>
<td>4.9 ± 1.1</td>
<td>4.1 ± 1.5</td>
<td>-16</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBF</td>
<td>27.4 ± 11.7</td>
<td>20.6 ± 4.9</td>
<td>-25</td>
<td><strong>0.04</strong></td>
<td></td>
</tr>
<tr>
<td>II WM</td>
<td>CBV</td>
<td>2.6 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>-9</td>
<td><strong>0.02</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MTT</td>
<td>6.6 ± 2.3</td>
<td>7.2 ± 1.5</td>
<td>10</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBF</td>
<td>67.3 ± 16.5</td>
<td>54.8 ± 16.6</td>
<td>-19</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>CBV</td>
<td>3.9 ± 0.1</td>
<td>3.3 ± 0.4</td>
<td>-15</td>
<td><strong>0.002</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MTT</td>
<td>3.7 ± 0.8</td>
<td>3.9 ± 1.1</td>
<td>7</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBF</td>
<td>CBV</td>
<td>MTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WM</td>
<td>26.9±8.4</td>
<td>20.3±5.5</td>
<td>-24</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2.6±0.2</td>
<td>2.3±0.3</td>
<td>-15</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBV</td>
<td>6.3±1.5</td>
<td>7.2±1.5</td>
<td>14</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>65.4±15.3</td>
<td>51.9±14.9</td>
<td>-21</td>
<td>0.0008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBV</td>
<td>3.9±0.2</td>
<td>3.3±0.4</td>
<td>-15</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT</td>
<td>3.8±0.9</td>
<td>4.2±1.4</td>
<td>10</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6.2 – Fractional rates of change of each parameter per month (i.e., the vertical scale is in units of month$^{-1}$) for CBF (left), CBV (middle) and MTT (right) for white
(top) and grey (bottom) matter. Rates of change that are significantly non-zero are marked by an asterisk (p values can be found in Table 6.3).

Table 6.3 – Fractional rates of change of each parameter per month in serial measurements. Statistically significant results are shown in bold. CBV declined at a statistically significant rate after treatment with bevacizumab. No other rates of change were significantly different from zero. Note that pre-treatment rates of change were only available in recurrent glioma patients, Group III, since only a single initial pre-treatment scan was available for each newly diagnosed patient.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>Group</th>
<th>Rate ± SE (range)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF / ml/100 g/min</td>
<td>WM</td>
<td>III pre</td>
<td>-0.016 ± 0.121 (-0.260 − 0.228)</td>
<td>0.8945</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III post</td>
<td>0.005 ± 0.036 (-0.066 − 0.076)</td>
<td>0.8916</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II post</td>
<td>0.083 ± 0.288 (-0.530 − 0.696)</td>
<td>0.7765</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I post</td>
<td>0.037 ± 0.031 (-0.024 − 0.099)</td>
<td>0.2294</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III pre</td>
<td>-0.161 ± 0.291 (-0.764 − 0.442)</td>
<td>0.5849</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III post</td>
<td>-0.048 ± 0.095 (-0.238 − 0.142)</td>
<td>0.6175</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II post</td>
<td>-0.317 ± 0.777 (-1.995 − 1.360)</td>
<td>0.6895</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I post</td>
<td>-0.022 ± 0.094 (-0.217 − 0.173)</td>
<td>0.8183</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III pre</td>
<td>-0.002 ± 0.003 (-0.008 − 0.003)</td>
<td>0.4393</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III post</td>
<td>-0.006 ± 0.002 (-0.010 − 0.002)</td>
<td>0.0033</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II post</td>
<td>-0.031 ± 0.009 (-0.050 − 0.011)</td>
<td>0.0052</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I post</td>
<td>-0.001 ± 0.001 (-0.003 − 0.001)</td>
<td>0.3506</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III pre</td>
<td>-0.002 ± 0.005 (-0.011 − 0.008)</td>
<td>0.7185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III post</td>
<td>-0.008 ± 0.003 (-0.014 − 0.003)</td>
<td>0.0056</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II post</td>
<td>-0.052 ± 0.016 (-0.087 − 0.017)</td>
<td>0.0065</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I post</td>
<td>0.000 ± 0.001 (-0.002 − 0.002)</td>
<td>0.6683</td>
</tr>
</tbody>
</table>
### Discussion

Post-treatment CBV and CBF were reduced in all groups receiving bevacizumab. CBV continued to fall for several months after treatment ended. Normal CBV and CBF values found in the recurrent glioma patients before bevacizumab treatment (Group III) and in other patients not receiving bevacizumab (Group I) suggest that standard treatments – surgery, cytotoxic chemotherapy and radiation – are not sufficient to cause this reduction. It therefore appears that these reductions are associated with treatment with bevacizumab. The cause of this is unclear. Turnover of endothelial cells in the brain is thought to be very slow so interference in this process is unlikely. It is possible that low levels of VEGF are necessary for the maintenance of normal function as in the kidney (92) and that bevacizumab disrupts this process. Nor is it clear why CBV should continue to decline months after treatment with bevacizumab has ended. However, speculation on mechanisms are beyond the scope of a brief report of a radiological finding. These are clearly matters for laboratory study.
No changes were seen in MTT suggesting that the reductions in blood flow directly reflect changes in volume and that there is no compensatory increase in blood velocity.

The introduction of anti-angiogenic therapy to the conventional treatment regimen for both newly diagnosed and recurrent HGG has shown a marked radiologic response of 50-80% with an improvement in quality of life secondary to the anti-edema effect (93,94). However, the increase in overall survival in glioma has been only moderate, of about 3 months. After VEGF blockade with bevacizumab, there has been a shift toward diffuse invasive relapse as a function of an especially hypoxic microenvironment, increasing the expression of factors that promote invasion (91,95-98). The relative absence of contrast-enhancing tumor compared with the non-contrast enhancing phenotype in many of the patients might serve to underscore the effect bevacizumab has on reducing vessel leakiness and thereby preventing contrast from entering areas with tumor as well as promoting tumor invasion. As a result, there is intense interest in imaging modalities like perfusion studies to help in predicting the response to therapy. This study suggests that care need be exercised in the use of relative CBV measurements (which use a reference in normal WM) to monitor treatment with bevacizumab and possibly other anti-angiogenic agents. Tumor relative CBV measurements are likely to underestimate initial reductions in blood volume after bevacizumab treatment. Furthermore the continued reduction in CBV in NAWM may suggest an apparent increase in blood volume in stable tumors.
There are several limitations to this study. First, there were small variations in imaging protocol across the subject group, in particular in flip angle. However, this will only have a large effect when contrast agent extravasation reduces $T_1$ significantly (99). Since measurements were made in NAWM where the blood brain barrier is intact we do not expect to see any major effects. Furthermore, there appeared to be no systematic association between a particular protocol and patient group so that any effects would appear as random noise rather than a systematic bias. Moreover, this limitation did not apply in Group III where only scans that used identical flip angles were analyzed.

Second, numbers of subjects, particularly in Groups I and II were small. However, the consistency of the results from these groups with the larger Group III, and the high statistical significance of, in particular, the CBV results give us confidence that the measurements are broadly correct. The heterogeneity of tumor grade within our patient group might also be seen as a limitation. However, if this effect were somehow dependent on grade, then this confounding factor would tend to reduce statistical significance rather than produce a false positive.
CHAPTER 7: Estimating Contrast Agent Concentration with Magnetic Field Correlation Imaging: A Future Direction

The work in this chapter appears in *Magnetic Resonance in Medicine* under the following citation:


This study was funded in part by NIH grants RO1CA093992 and RO1CA093992.

Author Contributions:

   Vishal Patil – Project concept and design, data acquisition and analysis, manuscript drafting and revisions.

   Glyn Johnson – Project concept and design, data acquisition, manuscript drafting and revisions.

   Jens H. Jensen – Project concept and design, data acquisition, manuscript drafting and revisions, final approval for publication.
Abstract

Contrast-enhanced perfusion studies of the brain by means of magnetic resonance imaging (MRI) are used to estimate a number of important brain tissue parameters, including cerebral blood flow and volume. In order to calculate these parameters, the contrast agent (CA) concentration must first be estimated. This is usually accomplished by measurement of a nuclear magnetic resonance (NMR) relaxation rate with the assumption of a linear relationship between the rate and the CA concentration. However, such a linear relationship does not necessarily hold in biological tissues due to compartmentalization of the CA in either the intravascular or extracellular spaces. Here we propose an alternative MRI method of CA quantification based on measurement of the magnetic field correlation (MFC), which is theoretically predicted to have a robust quadratic dependence on the CA concentration even when the CA is compartmentalized. In this study, CA concentration estimation by means of MFC is shown to be more accurate than established methods based on relaxation rates in yeast cell suspensions.
**Introduction**

Estimation of contrast agent (CA) concentration, $C$, is an essential first step in the measurement of a number of different brain tissue parameters, such as cerebral blood flow, cerebral blood volume and vascular transfer constant by contrast enhanced perfusion MRI. To accomplish this, a linear relationship between $C$ and the change in relaxation rate due to the presence of CA is usually assumed, i.e.

$$ R = R^{\text{tissue}} + rC $$  \hspace{1cm} (7.1)

where $R$ is the relaxation rate ($R_1$, $R_2$, or $R'_2$), $R^{\text{tissue}}$ is the relaxation rate in the absence of CA and $r$ is a constant expressing the relaxivity of CA (which in general is tissue dependent). However, it is known that this linear relationship is only strictly valid for simple solutions and may not hold in tissues due to compartmentalization of the CA in either the intravascular or extracellular spaces (35,51). As a consequence, the application of Eq. (7.1) to the analysis of contrast enhanced perfusion MRI data can lead to systematic quantification errors.

Another CA sensitive parameter is the magnetic field correlation (MFC), which may also be estimated with MRI (100,101). The MFC is defined by

$$ \text{MFC}(|t_2 - t_1|) = \gamma^2 \langle \delta B(t_1) \delta B(t_2) \rangle $$  \hspace{1cm} (7.2)

where $\delta B(t)$ is the local magnetic field shift (relative to the uniform background field) experienced by a water proton at a time $t$ and $\gamma$ is the proton gyromagnetic ratio. That the MFC is a function of the time difference, $|t_2 - t_1|$, rather than $t_1$ and...
$t_2$ individually is a consequence of time translation invariance. The dependence of
the MFC on the CA concentration is, to an excellent approximation (see Appendix
C), given by

$$\text{MFC}(t_2 - t_1) = a_1 (C + a_2)^2 + a_3$$  \hspace{1cm} (7.3)

where $a_1$, $a_2$, and $a_3$ are tissue specific parameters (that may also depend on the
time difference $|t_2 - t_1|$). The principal assumptions required for the validity Eq.
(7.3) are that the local magnetic field shift is much smaller than the applied field
strength and that the CA concentration can be regarded as constant during the
interval between $t_1$ and $t_2$. Since both of these conditions are well satisfied for a
typical MRI experiment, the accuracy of Eq. (7.3) is, in practice, likely to be higher
than that of Eq. (7.1). In particular, CA compartmentalization is expected to have a
much smaller effect on the accuracy of Eq. (7.3), than it does for Eq. (7.1).

The parameter $a_2$ is often relatively small and may be neglected. One can
then invert Eq. (7.3) to give

$$C \approx k \sqrt{\text{MFC} - \text{MFC}_0}$$  \hspace{1cm} (7.4)

where $k \equiv 1/\sqrt{a_1}$ and $\text{MFC}_0$ is the MFC for $C = 0$. Eq. (7.4) shows explicitly how
MFC measurements can be used to quantify a CA concentration up to an overall
tissue specific scaling factor.

The goal of this study is to test the accuracy of Eqs. (7.3) and (7.4) for yeast
cell suspensions by measuring the MFC for a range of concentrations of
gadolinium diethylenetriaminepentaacetic (Gd-DTPA). Yeast was used in the cell suspension phantom both for convenience and because their size and intrinsic properties mimic brain cells to some degree. Yeast cells are oblong shaped ranging from 7-10 µm in diameter, similar to microglial cells, and have $R_1$ (0.67 s\(^{-1}\)) and $R_2$ (12.3 s\(^{-1}\)) values which lie within the range of certain brain tissues (34,102-105). The accuracy of Eq. (7.1) for $R_1$, $R_2$ and $R_2^*$ relaxation rates is also assessed in the same yeast suspensions as a comparison.

**Methods**

Cell suspensions were prepared by mixing 63 g of yeast, Saccharomyces cerevisia, (Fleischmann’s Active Dry Baker’s Yeast) in 1 L of distilled water and allowing the mixture to settle for 48 hours at room temperature. After yeast activation, 500 mL of the supernatant was removed and the concentrated yeast suspension was mixed and aliquotted to six 60 mL plastic bottles. Gd-DTPA (Magnevist, Berlex Laboratories, Wayne, NJ) was added to each NMR bottle in varying amounts to yield concentrations of 0, 1, 2, 4, 7, and 10 mM. Six more 60 mL plastic bottles were filled with distilled water and the same concentrations of Gd-DTPA as the cell suspension bottles. MFC values are expected to be close to zero, and show no dependence on Gd concentration, for simple solutions. Bottles were shaken vigorously before imaging to ensure uniform distribution of cells and Gd-DTPA through the suspension. Six bottles at a time were submerged in a corn syrup bath to minimize susceptibility effects and imaged as a group. Corn syrup
was used because its extremely short $T_2$ eliminates its signal and any associated artifacts (100).

Imaging was performed on a Siemens 3 T Trio MRI scanner (Siemens Medical Solutions, NJ) with a multi-channel head coil. Single horizontal slices were acquired through the center of the array of bottles with segmented (multi-shot) EPI sequences to decrease imaging time. Common image parameters were as follows: $T_R = 2000$ ms; FOV $200 \times 200$ mm; slice thickness $1.7$ mm, matrix $128 \times 128$; EPI factor $13$; NEX 1.

Raw signal intensities ($S'$) in each bottle and mean background noise ($\sigma$) were measured within regions of interest (ROIs) using ImageJ 1.40 g (Wayne Rasband, National Institutes of Health, USA). Signal intensities were corrected for rectified noise using the formula

$$S' = \sqrt{S^2 + \sigma^2}$$  \hspace{1cm} (7.5)

where $S$ is the corrected signal intensity. MFC and relaxation time estimates were obtained by fitting the appropriate equations to the corrected signal intensities using non-linear least squares fitting routines (LAB Fit Curve Fitting Software, Silva).

MFC was measured with an asymmetric spin echo sequence with Hahn echo time $24$ ms, and refocusing pulse time shifts, $t_s = 0, -1, -2, -3, -4, -5, -6, -7$ and -8 ms (100). Negative values for $t_s$ indicate that the interval between the initial $90^\circ$ excitation pulse and $180^\circ$ refocusing pulse is reduced from the usual spin-echo
value of $T_e/2$). ROI signal intensities were corrected for noise (Eq. (7.5)) and fitted to Eq. (7.6).

It can be shown (100) that signal intensity is quadratically related to $t_s$

$$S = S_0 \exp(-2t_s^2 MFC) \quad (7.6)$$

MFC may then be estimated by a non-linear least squares fit of Eq. (7.6) to measured values of $S$.

$T_1$ measurements were performed using an inversion-recovery (IR) sequence with $T_e$ of 17 ms and 10 different inversion times, $T_I = 35, 55, 75, 95, 115, 200, 400, 600, 800, \text{ and } 1000 \text{ ms}$). Since magnitude reconstruction was employed negative ROI signal intensities appear positive. These were therefore corrected manually before correcting for noise. Corrected signal intensities were fitted to the following expression to estimate $T_1$.

$$S = S_0 \left(1 - 2e^{-T_1/T_s} + e^{-T_e/T_s}\right) \quad (7.7)$$

$T_2$ measurements were obtained using a spin-echo sequence with six different values of $T_e$ (17, 27, 37, 47, 57, and 67 ms). Similarly, $T_2^*$ measurements were made using a gradient echo (GRE) sequence with 12 different echo times $T_e$ (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 ms). Noise corrected signal intensities were then fitted to a simple monoexponential decays.

$$S = S_0 \exp \left(-\frac{T_e}{T_D}\right) \quad (7.8)$$
where $T_o$ is $T_2$ or $T_2^*$ for spin and gradient echo sequences respectively.

Eq. (7.3) was fitted to a plot of MFC vs. Gd concentration, $C$ by non-linear least squares fitting to yield estimates of $a_1$, $a_2$, and $a_3$. Estimated values of Gd concentration, $C_{est}$ were then obtained for each bottle using these values and the measured value of MFC. Similarly Eq. (7.1) was fitted to plots of $R_1$, $R_2$, and $R_2^*$ vs. $C$ by linear least squares fitting to yield estimates of relaxivities $r_1$, $r_2$, and $r_2^*$ and hence $C_{est}$ for each bottle.

Bland-Altman plots (i.e., plots of $C_{est} - C$ vs. $C_{est} + C/2$) were then generated to compare the agreement between true values of $C$, and estimates obtained by the MFC method and each relaxivity method.

**Results**

Typical measured signal intensities and fitted curves are given in Fig. 7.1. Fig. 7.2 gives plots of MFC and relaxation rates plotted against Gd-DTPA concentration ([Gd]) for both cell suspensions and water phantoms. MFC values were fitted to a quadratic model (Eq. (7.4)). This provided an excellent fit ($R^2 = 0.9991$) for the cell suspension (Fig. 7.2a). As expected, MFC values of the water phantoms are close to zero (-0.19-0.06) consistent with the absence of magnetic field inhomogeneities and show no variation with [Gd]. Figs. 8.7b-d gives plots of relaxation rate against [Gd] for both yeast and water phantoms. As expected relaxation rate varies linearly with [Gd] for the water phantoms (the dashed line
gives the linear least squares fit). However the response of the yeast phantom is
decidedly non-linear (the solid line is the fit through the first two data points for
illustration).

Fig. 7.3 illustrates the linear relationship between Gd-DTPA concentration Eq.
(7.4) $R^2 = 0.9956$. Fig. 7.4 shows Bland-Altman plots of MFC and relaxation
rates. The 95% limit agreements had ranges of 0.916 for MFC, 7.41 for $R_1$, 
2.35 for $R_2$ and 1.25 for $R_2^*$. 

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---

---
Fig. 7.1 – Cell suspension signal intensities with 4 mM Gd-DTPA versus a: the MFC refocusing pulse shift ($|t_1| \leq 8$ ms), b: $T_1$ in $T_1$ measurements ($T_1 \leq 1000$ ms), c: $T_E$ in $T_2$ measurements ($T_E \leq 67$ ms), and d: $T_E$ in $T_2^*$ measurements ($T_E \leq 60$ ms). The lines are fits to Eq. (7.6) (a), Eq. (7.7) (b) and Eq. (7.8) (c, d). Standard error estimates for all data points are shown with error bars.

Fig. 7.2 – MFC (a) and relaxation rates $R_1$ (b), $R_2$ (c) and $R_2^*$ (d) plotted against Gd-DTPA concentration for cell suspensions and water phantoms. MFC in the cell suspensions is fitted to a quadratic polynomial. In the relaxation rate plots, solid and
dashed lines represent a linear extrapolations, based on the $[\text{Gd}] = 0$ and $[\text{Gd}] = 1$ mM data points for the cell suspension and water phantom respectively. Standard error estimates for all data points are shown with error bars.

Fig. 7.3 – Plot of MFC difference as a function of Gd-DTPA concentration (Eq. (7.4)).
Fig. 7.4 – Bland-Altman plots for MFC (a) and relaxation rates $R_1$ (b), $R_2$ (c) and $R_2^*$ (d). The y axis represents the difference and the x axis the mean of estimated and true Gd concentrations respectively. The solid line indicates the mean difference and the dashed lines show ± 95% limit agreements (i.e., approximately twice the standard deviation of the difference).
Discussion

This study confirms that MFC measurements depend quadratically on Gd-DTPA concentration in yeast cell suspensions to a high degree of accuracy. This dependence is significantly more accurate than the generally assumed linear dependence of relaxation rates on concentration, supporting the potential of MFC to more accurately quantify CA concentrations. Comparison of the results from chapter 4 may not be appropriate since the contrast agent in this study was modeled to be extravascular and not intravascular.

A study by Tanimoto (2001) demonstrated a linear increase in an agar gel phantom between superparamagnetic iron oxide (SPIO) contrast agent concentration and relaxation rates ($R_1$, $R_2$, and $R_*^2$). However, this linear increase was not shown in Sephadex bead phantoms except for $R_*^2$ rates (52). Fig. 7.2 exhibits substantial deviations from linearity in the cell suspension phantom for relaxation rates in bottles with [Gd] over 7 mM. Also shown in Fig 7.2. is the strong quadratic relationship between MFC and Gd-DTPA at all concentrations up to 10 mM in a cell suspension.

Bland-Altman plots were used to compare each method of measuring [Gd]. These plots are preferable to comparing correlation coefficients a high correlation does not necessarily imply strong agreement (106). Fig. 7.4 shows that MFC and $R_*^2$ give the best agreement between estimated and true [Gd], with $R_1$, $R_2$ at least an order of magnitude worse. $R_*^2$ measurements are somewhat easier to make than
MFC measurements but the images are heavily susceptible to imaging artifacts due to macroscopic field inhomogeneities and low signal to noise ratios with increasing [Gd], both of which may be avoided by using MFC imaging (100).

The range of Gd concentrations used in this study is somewhat larger than would typically be found in tissues at equilibrium (an average of 0.1 mM with a standard dose) but might be seen during the first pass of a standard or multiple dose bolus. Moreover, errors in estimates are of similar size throughout the entire range of concentrations used here (Fig. 7.4).

This study used gadolinium diethylenetriaminepentaacetic (Gd-DTPA), a low-molecular weight (< 1000 Da) paramagnetic CA that is the most widely used contrast agent for human studies. However similar results are expected with other paramagnetic contrast agents and also those based on iron oxide particles. It should be noted that Gd-DTPA is water-soluble and homogeneous throughout the cell suspension. In this study, the distribution of Gd-DTPA was modeled after the extravascular extracellular space, not the intravascular space. Other factors that contribute to the complexity of estimating [Gd] are compartmentalization of CA, geometry of the compartments and the water diffusion rates associated with these compartments (34).

Finally, the effect that the errors in concentration estimates found here have on the accuracy of measured perfusion parameters is beyond the scope of this paper and is the subject of ongoing investigation.
CHAPTER 8: Conclusions and Future Directions

Conclusions

This thesis has presented:

1.) An analytical function which models the first-pass of a DSC MRI concentration-time curve that can be separated into bolus and recirculation components. Most previous studies model the first-pass by a gamma variate which does not account for recirculation and requires a method for determining the last point to fit. These limitations cause inaccuracies and imprecise estimates of the area under the curve and first moment of the bolus in the presence of noise. However, the SCR model accurately describes the fist pass plus recirculation and does not require determination of the last point to fit since it fits the entire concentration-time curve. The SCR model is less affected by noise.

2.) Empirically determined relaxivity functions in tissue. The results in chapter 4 confirm that using a linear relaxivity function for quantifying tissue concentration decreases both accuracy and precision in perfusion estimates. In contrast, the empirically derived nonlinear function yields precise and accurate estimates over a range of field strengths and echo times.

3.) Empirically determined relaxivity functions in venous blood. *In vitro* experiments using bulk blood phantoms provide useful calibration relaxivity curves for arterial blood. However, similar experiments with venous blood provide
anomalous results. Specifically, an initial decrease in relaxivity as contrast agent concentration increases which is not seen in vivo. Using the fitting procedure devised for tissue, we were able to provide accurate relaxivity functions for venous blood that did not show this anomaly (Chapter 5)

4.) An observation of the reduction of perfusion in normal appearing tissue in brain cancer patients treated with bevacizumab. Typically, normal appearing tissue contralateral to the tumor is used as a reference tissue when making relative CBV measurements. The study conducted in chapter 6 provides strong evidence that perfusion in the normal appearing tissue of patients treated with bevacizumab is reduced and thus that relative measurements must be treated with caution.

4.) In vitro demonstration of the accuracy of a new imaging technique, MFC, in estimating contrast agent concentration. The major advantage of MFC over traditional relaxation rate measurement is that MFC dependence on concentration does not depend on contrast agent compartmentalization (i.e., intra- or extra-vascular).

Future Directions

There still remain a number of issues which must be addressed before DSC MRI is considered the “gold standard” for measuring brain perfusion.

First, although the SCR model appears empirically to match actual concentration measurements, it has not theoretical justification. It may be possible to derive theoretically expressions that take into account i) second pass effects, ii)
the exchange of contrast between intra- and extra-vascular spaces that occur before contrast arrives in the brain and iii) the different path lengths of contrast passing through different vascular pathways.

Second, while we have identified better empirical relaxivity functions for tissue and venous blood, discrepancies between experimental and theoretical results indicate that a complete biophysical explanation of relaxivity has yet to be fully determined. Future work may directly model the competing contributions to susceptibility contrast (RBC oxygenation, plasma susceptibility, contrast agent effects, and vascular geometry) to better understand or improve the nonlinear relaxivity functions and their theoretical justifications. However, the new relaxivity functions could now be used to recalibrate clinical perfusion thresholds for different diagnoses and prognoses.

Third, the relaxivity of extravasated blood must be determined to estimate contrast agent concentration in areas which are affected by damage to the blood-brain barrier. MFC is a promising method for this purpose since compartmentalization does not affect its dependence on concentration. Two different studies must be performed before MFC can be used for DSC MRI. First, MFC should be measured before and during the recirculation phase of DSC MRI experiments (the biological half-life of Gd-DTPA is approximately 7 min to calibrate the tissue specific parameter, $a_1$, in normal and abnormal tissue. Second, a dynamic MFC measurement sequence with 1s temporal resolution sequence must be developed and tested. Results can then be compared against other perfusion
techniques. If successful, MFC may be the first perfusion MRI technique able to quantify contrast agent concentration accurately in both normal tissue and tissue suffering from a damaged blood-brain barrier.

Fourth, the rationale for finding accurate relaxivity functions for venous blood is that venous measurements, being acquire from large vessels, might be used to correct for partial volume effects on AIF estimates. This has yet to be demonstrated.

Fifth, this thesis mainly focused on the errors in CBV but the errors in CBF and MTT should also be examined.

Finally, this thesis has concentrated on DSC MRI, however dynamic contrast-enhanced (DCE) MRI is an alternative analysis that takes into account vascular permeability. Although DCE MRI usually uses T1-weighted data, similar analyses may be performed on T2*-weighted data. The implications of using improved relaxivity functions with DCE MRI analyses have yet to be studied.

At its inception in the late 1980s, the main criticism of DSC MRI was that, relative to its major rival, arterial spin labeling, it was “non-quantitative”. However, since then DSC MRI has been continuously. Nonetheless, DSC MRI is, without question, the most commonly used perfusion MRI technique for cerebral imaging in the clinic. With improvements in image quality, better techniques for acquiring the AIF and improved relaxivity and analysis methods such as those presented in this thesis, DSC MRI may be on the verge of acceptance as a fully quantitative method.
APPENDIX A

The following is a mathematical proof of Eq. (1.3), $V = FM$, when using an instantaneous injection (note: a constant infusion – indicator being introduced continuously at a constant rate – will not be considered) (11,32,107). Let $q$ be the quantity of indicator injected at the beginning of a system and $c(t)$ is the concentration of indicator at the end of the system. During the time interval $t$ to $t + dt$ the amount of indicator that has left the system is the volume – which can be expressed as $Fd t$ multiplied by $c(t)$. Since the total amount of indicator entering the system must also equal the amount leaving the system

$$q = \int_0^\infty c(t) F dt$$  \hspace{1cm} (A.1)

and $F$ can be calculated by rearranging Eq. (A.1)

$$F = \frac{q}{\int_0^\infty c(t) dt}.$$  \hspace{1cm} (A.2)

The rate at which indicator leaves the system can be expressed as the product of $F$ and $c(t)$ and can be modeled as a distribution function, $h(t)$, which describes the distribution of transit times of the indicator in the system normalized to the amount of indicator.
\[ h(t) = \frac{Fc(t)}{q} = \frac{c(t)}{\int_0^\infty c(t) \, dt}. \]  

(A.3)

Since \( h(t) \) is a distribution function it must follow the following property,

\[ \int_0^\infty h(t) \, dt = 1. \]  

(A.4)

(Introducing \( h(t) \) allows us to quantify indicator in the system as a concentration and not a finite quantity.) The rate at which the indicator enters and leaves the system can be expressed as \( Fh(t) \, dt \) and the volume of indicator is the time it takes, \( t \), to leave the system multiplied by the rate,

\[ dV = tFh(t) \, dt \]  

(A.5)

so that the volume of the system, \( V \), is

\[ V = \int_0^\infty tFh(t) \, dt = F \int_0^\infty th(t) \, dt. \]  

(A.6)

Because \( h(t) \) is a distribution function of the transit times it should be noticed that \( \int_0^\infty th(t) \, dt \) is the first moment,

\[ M = \int_0^\infty th(t) \, dt \]  

(A.7)

and therefore proving the central volume theorem.

In practice, achieving an ideal instantaneous injection where the total amount of indicator can be introduced into the system at time zero is not practical. If \( M \) is much greater than the injection time then the above proof holds, however if the injection time is comparable to \( M \) then the observed \( M \) will be greater than
the true $M$. The following modifications to the proof must be made: First, the injection can be considered to be a function continuous series of instantaneous injections, $C_i(t)$, and each injection is superimposed by a tissue response function, $R(t)$, known as the residue function. The resulting distribution of transit times can be expressed as a convolution.

$$h(t) = \int_0^t C_i(\tau) R(t-\tau) d\tau$$  \hspace{1cm} (A.8)

Combining Eqs. (A.4) and (A.8) gives,

$$\int_0^\infty h(t) dt = \int_0^\infty \int_0^t C_i(\tau) R(t-\tau) d\tau dt = 1$$  \hspace{1cm} (A.9)

and by the additive property of the first moments of convolved function,

$$M = \int_0^\infty t h(t) dt = \int_0^\infty t C_i(t) dt + \int_0^\infty t R(t) dt$$  \hspace{1cm} (A.10)

Eq. (A.7) is proven for an injection whose injection time is comparable to $M$.  

110
APPENDIX B

The following is a mathematical proof of Eq. (1.10), \( \text{MTT} = \int_{0}^{\infty} R(t) dt \).

The definition of \( R(t) \) is

\[
R(t) = 1 - \int_{0}^{\infty} h(t) dt
\]

(B.1)

so that

\[
h(t) = - \frac{dR}{dt}.
\]

(B.2)

Combining Eqs. (A.7) and (B.2),

\[
\text{MTT} = - \int_{0}^{\infty} t \frac{dR}{dt} dt
\]

(B.3)

and integrating \( - \int_{0}^{\infty} t \frac{dR}{dt} dt \) by parts,

\[
\text{MTT} = \int_{0}^{\infty} R(t) dt - \left[ Rt \right]_{0}^{\infty}.
\]

(B.4)

Since the amount of indicator injected is finite and by definition, again, \( R \) is the amount of indicator remaining in tissue at time, \( t \), \( \lim_{t \to \infty} R(t) = 0 \), thus proving Eq. (1.10).

The following is a brief explanation for Eq. (1.12), \( \text{CBV} = \frac{k_{H}}{\rho} \int C_{i}(t) dt \int C_{a}(t) dt \).

By the principle of conservation of mass and using Eq. (A.1),
Eq. (1.12) is then obtained by rearranging Eq. (B.5)
Here we sketch the derivation of Eq. (7.3). If the CA is distributed according to a fixed spatial pattern, then the local magnetic field shift for an individual water proton depends linearly on the CA concentration so that

\[ \delta B(t) = \delta B'(t) + C \cdot F(t) \]  

(C.1)

where \( \delta B'(t) \) is the field shift in the absence of the CA and \( F(t) \) is a function that depends on the water proton's diffusion path, and where we have assumed a time-independent concentration. The validity of Eq. (C.1) also requires that \( \delta B(t) < B_0 \) and \( \delta B'(t) < B_0 \), with \( B_0 \) being the applied field, but these two conditions are generally well satisfied for clinical scanners. From Eqs. (7.2) and (C.1), one finds

\[
\begin{align*}
\text{MFC}(t_2 - t_1) &= \gamma^2 \langle \delta B'(t_1) \delta B'(t_2) \rangle + \\
&+ \gamma^2 C \langle \delta B'(t_1) F(t_2) + F(t_1) \delta B'(t_2) \rangle + \gamma^2 C \langle F(t_1) F(t_2) \rangle
\end{align*}
\]

(C.2)

The identifications

\[
\begin{align*}
a_1 &= \gamma^2 \langle F(t_1) F(t_2) \rangle \\
a_2 &= \frac{1}{2a_1} \gamma^2 C \langle \delta B'(t_1) F(t_2) + F(t_1) \delta B'(t_2) \rangle \\
a_3 &= \gamma^2 \langle \delta B'(t_2) \delta B'(t_1) \rangle - a_1 a_2^2
\end{align*}
\]

(C.3)

then lead directly to Eq. (7.3).
REFERENCES


31. Stewart GN. The pulmonary circulation time, the quantity of blood in the lungs and the output of the heart. American Journal of Physiology 1921;58(1):20-44.


