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Blood Oxygen Level Dependent Angiography (BOLDangio) and Its Potential Applications in Cancer Research

Kejia Cai^{a,*}, Adam Shore^a, Anup Singh^a, Mohammad Haris^a, Teruyuki Hiraki^b, Prianka Waghray^c, Damodar Reddy^a, Joel H. Greenberg^b, and Ravinder Reddy^a

^aCMROI, Department of Radiology, University of Pennsylvania, Philadelphia, PA

^bDepartment of Neurology, University of Pennsylvania, Philadelphia, PA

^cDepartment of Biology, Wake Forest University, Winston-Salem, NC

Abstract

Clinically, development of anti-angiogenic drugs for cancer therapy is pivotal. Longitudinal monitoring of tumor angiogenesis can help clinicians determine the effectiveness of anti-angiogenic therapy. Blood Oxygen Level Dependent (BOLD) effect has been widely used for functional imaging and tumor oxygenation assessment. In this study, the BOLD effect is investigated under different levels of oxygen inhalation for the development of a novel angiographic MRI technique, Blood Oxygen Level Dependent angiography (BOLDangio). Under short-term (<10 min) generalized hypoxia, which is induced by the inhalation of 8% oxygen, we measure BOLD contrast as high as 25% from vessels at 9.4T using a simple gradient echo (GRE) pulse sequence. This produces high resolution 2D and 3D maps of normal and tumor brain vasculature in less than 10 minutes. Additionally, this technique reliably detects metastatic tumors and tumor induced intracranial hemorrhage. BOLDangio provides a sensitive research tool for MRI of vasculature under normal and pathological conditions. Thus, it may be applied as a simple monitoring technique for measuring the effectiveness of anti-angiogenic drugs in a preclinical environment.

Keywords

blood oxygen level dependent angiography; tumor angiogenesis; vasculature; tumor metastasis; intracranial hemorrhage; MRI

Introduction

Angiogenesis, the physiological process that leads to the growth of new blood vessels, has many roles in the human body. Its primary role in healthy individuals is the generation of new vasculature to enhance oxygen and nutrient exchange, providing a necessary step in the development and growth of new tissues. Unfortunately, angiogenesis also plays a fundamental role in tumor growth and metastasis (1, 2). Tumor vasculature developed via angiogenesis, in particular, is characterized by immature, fragile, and chaotic structures. This can cause local tissue hypoxia that effectively starves the surrounding healthy tissue of vital nutrients (1, 2). Luckily, a reduction in tumor angiogenesis has been shown to decrease tumor size and lower a patient's risk of metastasis (2, 3). As a result of this effect, both anti-

*Address for Correspondence, Kejia Cai, PhD, Center for Magnetic Resonance and Optical Imaging (CMROI), Department of Radiology, University of Pennsylvania, B1 Stellar-Chance Laboratories, 422 Curie Boulevard, Philadelphia, PA 19104-6100, Tel: (215) 898-9357, Fax: (215) 573-2113, kcflying@gmail.com.

angiogenic drugs and angiogenic inhibitors have become an increased area of focus for researchers. Endostatin and Avastin, for example, are antiangiogenic agents that have been proven to inhibit tumor growth by reducing tumor angiogenesis (4).

In order to further understand the relationship of anti-angiogenic agents on cancer progression, researchers require the ability to accurately quantify the amount of tumor vasculature present at each stage of treatment. Therefore, a cost-efficient method must be developed to image tumor vasculature in order to determine treatment efficacy in terms of the inhibition of tumor growth and the reduction of the risk of metastasis. Since changes in tumor size usually lag behind changes in angiogenesis, imaging tumor angiogenesis and vasculature may provide early detection of tumor development and metastasis.

Several angiography techniques are available for imaging vasculature in small animals. Contrast enhanced CT imaging successfully uses exogenous contrast agents to correlate physiological markers (such as tissue perfusion parameters, microvascular density, and vascular endothelial growth factor (VEGF)) with tumor angiogenesis but exposes the subject to ionizing radiation (5). Studies using microCT generate 3D maps of mouse brain vasculature by filling the circulatory system with radio-opaque silicone rubber (6). Unfortunately, this prevents the longitudinal investigation of vasculature pathology as the procedure necessitates animal sacrifice. An improved optical coherence tomography (OCT) technique, called optical frequency domain imaging (OFDI) (7) has also been developed for extremely high resolution imaging of tumor angiogenesis; however, the penetration depth is limited to the mm range. MRI, on the other hand, does not suffer from the same limitations as the aforementioned techniques as it can produce high resolution images without either the use of ionizing radiation or the limitations of optical penetration. Many of the current MRI angiographic techniques employ exogenous contrast agents. Dynamic contrast-enhanced (DCE)-MRI (8), for example, provides a way of directly measuring angiogenesis with the injection of Gadolinium (Gd) based paramagnetic agents, Gd loaded nanoparticles (9), or iron oxide nanoparticles. Regrettably, the resolution of vasculature imaging with these techniques is often reduced due to the enhanced tumor permeability and the leakage of contrast agent from the circulation (10). Time of flight (TOF) MRI angiography, based on the contrast between partially saturated static tissue and unsaturated flowing blood, can also be used for small animal angiography in tumor (10) and focal cerebral ischemia models (11–13). Using this technique to detect small angiogenic vessels, however, is quite challenging due to slow blood flow rates and partial saturation of the blood signal (10–13). Additionally, the vessel signal shown in most TOF images is not normalized to a baseline and thus cannot be used quantitatively. Therefore, there is still a need for an alternative, sensitive and quantitative imaging technique for tumor vasculature MRI.

Using the simplest gradient echo pulse sequence (GRE), the Blood Oxygen Level Dependent (BOLD) effect is measured by quantifying the reduction in signal that results from the phase dispersion of the water proton signal caused by changes in paramagnetic deoxyhemoglobin (dHb). Presently, BOLD MRI has emerged as the dominant technique for performing functional MRI (14, 15), measuring vasculature function and maturation (16), and detecting oxygen saturation changes (17, 18).

In this study, we carefully investigate the BOLD effect under different levels of oxygen inhalation in order to develop a novel MRI angiographic technique, hereafter called Blood Oxygen Level Dependent angiography (BOLDangio). BOLD contrast generated under hypoxia or hyperoxia is experimentally determined and verified with blood oxygen saturation (SO_2) and R_2^* relaxation rate measurements. Hypoxia is shown to have much greater capacity for generating high resolution 2D and 3D BOLD angiograms that suggest the potential to monitor anti-angiogenic drug therapies, identify metastatic tumors, and

detect tumor induced hemorrhage. A comparison of BOLDangio with Gd perfusion angiography is also presented.

Theoretical Considerations

Generally, MRI GRE signal is determined by

$$S = A * e^{-TE * R_2^*} \quad (1)$$

where TE is the echo time, R_2^* (or $1/T_2^*$) is the T_2^* relaxation rate. Parameter A is a function of proton density, T_1 relaxation time, and flip angle of RF pulse (19). As a paramagnetic contrast agent, deoxyhemoglobin concentration [dHb] has been shown to have an approximately linear effect on blood R_2^* (17, 20, 21) as indicated in Equation 2:

$$R_2^* = R_{2,0}^* + r * [dHb] \quad (2)$$

where $R_{2,0}^*$ is the relaxation rate of “whole blood” in the absence of dHb and r is the relaxivity constant of dHb. Rearranging, the change in R_2^* is shown to be directly proportional to the change in [dHb].

$$\Delta R_2^* = r * \Delta [dHb] \quad (3)$$

As it is well known that

$$[dHb] = \text{Blood Density} * \text{Hct} * SO_2 \quad (4)$$

and blood density and hematocrit (Hct) in vessels are independent of blood oxygen saturation level (SO_2) changes, changes in dHb concentration can be written as

$$\Delta [dHb] = \text{Blood Density} * \text{Hct} * \Delta SO_2 \quad (5)$$

Substitution into Equation 3 reveals a linear relationship between changes in oxygen saturation (SO_2) and changes in R_2^* :

$$\Delta R_2^* = B * \Delta SO_2 \quad (6)$$

where $B = r * \text{Blood Density} * \text{Hct}$. This concept has been widely used for quantifying tissue and blood oxygenation with BOLD effect based MRI (18).

BOLD contrast is determined by calculating the percent signal difference between inhalation 1 (either hyperoxia or hypoxia) and inhalation 2 (normoxia) with Equation 7.

$$\text{BOLD\%} = 100\% * \frac{|S_1 - S_2|}{S_2} \quad (7)$$

Combining with Equation 1, it can be written as

$$\text{BOLD\%} = 1 - e^{-TE * |R_{2,2}^* - R_{2,1}^*|} \quad (8)$$

Substituting in Equation 6, we end with a representation of BOLD contrast in terms of changes in blood oxygenation:

$$\text{BOLD}\% = 1 - e^{-TE \cdot B^* \Delta SO_2} \quad (9)$$

As a result, the BOLD contrast of arterial and venous vessels due to inhalation challenges is mainly dependent on oxygenation saturation differences assuming that there is very little change in proton density, blood density, Hct and T_1 values of arteries and veins as previously reported (21–25).

Materials and Methods

Tumor Model Preparation

To develop intracranial tumors, rat gliosarcoma cells (9L) were injected into Syngeneic female Fisher rats (F344/NCR, four-six weeks old) weighing 130–150 grams as described previously (26, 27). Briefly, general anesthesia was induced by isoflurane followed by intraperitoneal injection of a ketamine/acepromazine mixture at a dose of 91/9.1 mg/kg. A 10 μ l suspension of 50,000 9L cells in phosphate buffered saline was injected into the cortex at a depth of 2mm with a Hamilton syringe and a 30-gauge needle using a stereotactic apparatus (3mm lateral and 3mm posterior to the bregma). Four to six weeks after tumor cell implantation, rats were subjected to MRI studies.

Animal Preparation and Imaging

Rats with (n=4) and without (n=4) brain tumors were anesthetized with isoflurane (3% for induction, 1.5% maintenance) and imaged on a 9.4T horizontal bore small animal scanner (Varian, Palo Alto, CA) with a 35-mm diameter commercial quadrature proton coil (m2m Imaging Corp., Cleveland, OH). Animals were kept under anesthesia (1.5% isoflurane in oxygen) and their body temperatures were maintained (37 ± 1 °C) with the air generated from a heater (SA Instruments, Inc., Stony Brook, NY). Respiration and body temperature were monitored using a MRI compatible small animal monitoring system (SA Instruments, Inc., Stony Brook, NY).

T_2^* -weighted images were acquired from rats under different levels of inhaled oxygen (8%, 15%, 21%, and 100%), diluted with nitrogen, at a fixed flow rate of 35 cc/min. Gas mixtures were administered to the animals through a long tube (~10 m) that was connected to two separate gas tanks (pure oxygen or nitrogen) via a Y-shaped connector. Each gas tank was regulated with a flow meter that was calibrated using the water displacement method. The flow rate used was higher than the normal flow rate for rats, ~20 cc/min (28), to ensure that oxygenation adjustments were carried out instantly through the nose cone. Each gas mixture was administered for 3 min to allow the respiration rate to reach a steady state before the start of the MRI study. T_2^* weighted images of the rat brain in an axial view were acquired using a 2 dimensional (2D) multi-slice GRE pulse sequence with parameters: field of view = 35 $\times$ 35 mm², number of slices = 20, slice thickness = 0.5mm, matrix size = 256 $\times$ 256, flip angle = 20°, TR = 500 ms, TE = 5, 10, 15 and 20 ms, number of averages = 1. 3D vasculature maps at a single TE were generated using a pair of oxygen inhalations each given for ~5 min (2 min of imaging time and 3 min of preparation). R_2^* maps were additionally generated by linearly fitting the natural logarithm of the MR signal versus its corresponding TEs, as can be derived from Equation 1.

After oxygen inhalation studies, gadolinium-based perfusion angiography of two tumor rats were performed. Multi-slice T_1 -weighted GRE images were acquired before and immediately after the intravenous injection of an initial bolus of Gadopentetate dimeglumine (0.5 mmol Gd/ml; 0.15ml; Magnevist, Schering, Berlin, Germany) using the sequence determined shortest TE/TR (4.9/142.9 ms) to minimize the acquisition time (~24s). Other

parameters were: field of view = $35 \times 35 \text{ mm}^2$, number of slices = 15, slice thickness = 0.5 mm, matrix size = 256×128 , interpreted into 256×256 , flip angle = 20° .

Image Processing

Image processing and data analysis were performed using MATLAB (version 7.5, R2007b, Mathworks, Natick, Massachusetts, USA) and ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA). Multi-slice image volumes were co-registered in ImageJ using the “MultiStackReg” routine and “affine” algorithm to remove motion artifacts (29). Multi-slice BOLD contrast maps were then constructed in MATLAB. The resulting multi-slice BOLD maps were interpolated to generate isotropic voxels of volume $0.14 \text{ mm} \times 0.14 \text{ mm} \times 0.14 \text{ mm}$ to generate a 3D maximum intensity projection (MIP) image in ImageJ, where MIP images were color-mapped as “red hot”. The same image processing methods used above were applied to the Gd based angiography, for which Gd induced contrast maps were generated using co-registered datasets acquired before and post Gd injection.

MR signal, R_2^* relaxation rate, and percentage BOLD effect were quantified and averaged from manually selected regions of interest (ROIs). Vessel ROIs were taken from visible vessels (Figure 1C arrows) while cortical brain tissue were taken where no apparent vessels were visible in the 8% hypoxia images. Data was transferred between ImageJ and MATLAB using a shared “dicom” or “raw” formats.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using MATLAB to compare the differences in BOLD contrast between vessels and brain tissue due to inhalation challenges. Values are reported as mean $\pm$ standard deviation. Group differences were considered to be significant at $p < 0.05$.

Blood Gas Measurement

Due to setup difficulties, monitoring the physiological parameters including blood pressure, heart rate, respiration rate and blood gas measurements of arterial and venous blood was done out of magnet with a separate batch of age-matched control rats ($n=4$). Animals were prepared identically as for the imaging studies. Body temperature was monitored by a rectal probe and maintained at $37 \pm 1^\circ \text{C}$ with a heating pad (ATC1000, World Precision Instruments, Sarasota, FL). The femoral artery and vein were cannulated with a polyethylene catheter (PE-50) for the measurement of arterial pressure and for blood sampling. Arterial blood pressure was monitored using a pressure transducer on a computer based recording system (PowerLab, ADInstruments, Colorado Springs, CO). Animals were administered increasingly lower mixtures of inhaled oxygen diluted with nitrogen, 100%, 60%, 21% and 8% O_2 . After 5–6 minutes under each type of inhalation, blood samples were taken from the femoral artery and vein catheters and measured with a blood gas analyzer, i-STAT (Abbott Laboratories, Abbott Park, IL).

Results

When animals were subjected to different percentages of inhaled oxygen, the GRE signal intensity observed from both arterial and venous blood vessels changed in conjunction with blood oxygenation. As shown in Figure 1A–B, there was no obvious MR signal and R_2^* difference between either the blood vessels or the brain tissue when rats were exposed to hyperoxia inhalation (100% O_2). However, under increasingly hypoxic inhalation (15 - 8% O_2), there was a dramatic decrease in vasculature signal due to the BOLD effect (Figure 1C). The hypo-intense stripes (arrows) in the cortex correspond to cortical veins and arteries.

The relative signal ratio between cortical brain tissue and vasculature ($S_{\text{brain}}/S_{\text{vessel}} \pm \text{standard deviation}$) was 1.03 ± 0.06 , 1.03 ± 0.04 , 1.07 ± 0.2 and 1.21 ± 0.10 at 100%, 21%, 15% and 8% O_2 respectively (TE = 10ms). The corresponding BOLD contrast map (Figure 1D) clearly showed normal brain vasculature only with induced inhalation of 8% O_2 and not with either 15% or 100% O_2 . Blood gas measurements (Figure 1E) demonstrated increased oxygen saturation (% SO_2) as administered inhaled oxygen percentage increased. The difference in blood oxygen saturation between 21% and 8% oxygen were similar in artery and vein (39.5% vs. 47.0%), yielding similar BOLD contrast from artery and vein *in-vivo* based on Equation 9.

Just as Figure 1 shows a decrease in MR T_2^* weighted signal as a function of oxygenation, Figure 2.A shows the corresponding mathematical trend in R_2^* of vessels and brain tissue under decreasing levels of inhaled oxygen. Based on the difference of R_2^* , BOLD effects are calculated and shown in Figure 2.B, where hypoxia gas of 8% O_2 induced much higher BOLD contrast than 15% O_2 . A statistically significant difference in BOLD contrast between vessel and brain tissue is only observed under 8% O_2 (* $p < 0.05$). Experimental BOLD effects from vessels induced with 8% O_2 ($25.9 \pm 1.5\%$) is similar to the calculated BOLD contrast based on R_2^* differences (Figure 2.B). BOLD contrast as a function of TE is shown in Figure 2.C. The ratio between vessel contrast and brain tissue contrast remains about the same as TE increases from 5 to 20ms.

T_2^* weighted GRE images of normoxic and hypoxic (15% and 8%) as well as corresponding BOLD contrast maps of a brain tumor are shown in Figure 3 and revealed a well-defined ring of tumor angiogenic vasculature at the tumor rim (Figure 3). Based on the histograms of pixels from the tumor rim, most of the pixels showed BOLD contrast over 20% when induced with 8% oxygen, while <15% when induced with 15% oxygen. As shown in Figure 4, two metastatic tumors are observed at the edge of the primary tumor (the red circle), where tumor cells were initially implanted. Hypointense signal from intracerebral hematoma was also observed in the GRE image corresponding to normoxic inhalation (red arrows at Figure 4A). Unlike regular tumor vasculature, increasingly hypoxic inhalation does not further decrease signal from the hematoma as confirmed by low BOLD contrast (Figure 4.C).

As shown in Figure 5, a MIP rendering of the brain vasculature can be readily obtained with a multi-slice GRE acquisition. Normal brain vasculature was symmetrically distributed across the brain while tumor brain vasculature was abnormal and showed a bird-cage like structure surrounding tumor. Additionally, a metastatic tumor was visible at the bottom of the primary tumor. The tumor vasculature was seen to be fed from multiple pre-existing large vessels at the bottom of the tumors. The path of these pre-existing vessels seemed to be altered by the presence of tumor vasculature. Overall, the MIP map not only showed the tumor vasculature but also its origins from larger vessel alternation. Lastly, BOLD angiography images were validated with a Gd-based angiography technique (Figure 6) by showing similar vasculature distributions from each technique.

Discussion

There is a clear need for a less invasive, high resolution, sensitive and quantitative imaging technique for tumor vasculature research. The use of short term hypoxia suggests a novel technique for generating vasculature images based on the BOLD effect. As shown in Figure 1, when a rat is exposed to 8% oxygen, there is a dramatic decrease in the vessel signal due to the BOLD effect. As the inhalation oxygen level is decreased, blood oxygen saturation decreases, and dHb concentration increases. Oxygen saturation changes about the same amount for arterial and venous blood between 8% and 21% of oxygen inhalations, leading to

a similar BOLD effect from arterial and venous vessels as indicated in Equation 9. The change of blood SO_2 and R_2^* due to hyperoxia is much smaller compared to hypoxia. As a result, the BOLD effect induced by hyperoxia is much smaller. These results suggest that hypoxia inhalation is necessary to visualize the vasculature using BOLD contrast.

It is important to note that the ROI selection of normal brain tissue may be subject to partial volume of vessels and vice versa, which also leads to a BOLD effect from brain tissue. Higher resolution acquisition can help to better distinguish vessel boundaries for reducing partial volume effect, while requiring longer imaging time to maintain a similar signal to noise ratio. During ROI selection of vessels, we avoided vessels with large diameter because blood flow and motion artifacts may affect the accurate determination of relaxation rate of especially large vessels (30, 31). Vessel dilation occurs during hypoxia (32). Given that <20% of diameter changes are expected with inhalation of 8% oxygen (32) and that the similar signal level between brain tissue and vessels seen under normoxia, we believe that vessel dilation did not significantly affect BOLDangiograms.

TE is expected to play another factor affecting the amount of BOLD contrast. As shown in Equation 9 and in Figure 2.C, as TE increases, a higher level of BOLD contrast is expected. However, under the experimental range of R_2^* changes, the TE dependence curves are close to linear. This means that the ratio of BOLD contrast between vessels and brain tissue remains nearly constant for all TEs. It is also well known that increasing TE leads to exponentially decreased signal to noise ratio and a greater vulnerability to static field inhomogeneity. To mitigate these concerns a relatively short TE (10ms) was chosen for most of our experiments as a compromise. Additional optimizations for specific cases may be needed.

In choosing the amount of oxygen needed to generate the BOLD contrast, it is important to do so without harming the subject under study. In some studies, oxygen inhalation levels as low as 6% to 10% have been used for healthy human subjects and patients (34, 35). While, in small animals, acute and chronic administration of oxygen gas as low as 8% has been widely used in pathological and imaging studies. Some animals are able to live in 8% O_2 from hours to even days (36–41). Based on our observation, rats under anesthesia are able to adapt to 8% O_2 in a couple of minutes and reach a stable respiration, heart rate and blood pressure. It is necessary, however, to investigate if a less hypoxic amount of oxygen can induce sufficient BOLD effect from vessels so as to make this procedure safer and more widely applicable. As shown in Figures 1 to 3, however, 15% O_2 could not provide sufficient BOLD contrast for vasculature mapping and so this technique will probably be limited to translational research with small animals.

While the application of BOLDangio is applicable to normal brain vasculature, it is also highly sensitive to tumor vasculature resulting from tumor angiogenesis, as demonstrated in Figure 4–6. As previously discussed, tumor angiogenesis forms a region of high density vasculature which is asymmetrically distributed in the tumor rim (42, 43). The same pattern is seen from Figure 4 and other previous reports (43–45).

Since the development of metastatic tumors is initialized by angiogenesis process (46), tumor vasculature imaging could detect metastatic tumors at a very early stage. This could be vitally important to a cancer patient, because metastasis, or cancer mitigation, is usually an indicator of cancer aggressiveness. Direct imaging of tumor metastasis induced angiogenesis has been rarely reported especially in animal models (47–49). In this study, we clearly demonstrate that BOLDangio can detect the angiogenic vasculature associated with metastatic tumors from 9L gliosarcoma, an aggressive model (26, 27, 50).

Although our technique is specifically designed to image angiogenic tumor vessels, BOLDangio is a non-selective process that is capable of visualizing all kinds of brain vasculature anomalies, including hemorrhage. Various types of brain tumors may cause hemorrhage from ruptured blood vessels. The extravasation of blood out of the circulation can a mass called hematoma, which could potentially damage the nearby brain tissues (51). In this study, hypointense signal from an intracerebral hematoma was observed in the GRE images was seen even under normoxic inhalation (Figure 4), as confirmed by others (52–54). Thus, switching to hypoxic inhalation did not alter the signal from the hematoma, demonstrating that BOLDangio can serve as a reliable tool for the detection of hematoma and the clear differentiation of a hematoma from a functional vessel cluster.

2D multislice GRE imaging sequence used in this study can provide us 3D vasculature imaging of rats in several minutes, the same duration required for a single slice image. Symmetric and healthy distribution of normal rat vasculature and a distinct tumor vasculature network are seen from normal and tumor rat respectively. Instead of using a multi-slice 2D GRE sequence, a 3D GRE sequence could provide us better signal to noise ratio. However, the imaging time with a 3D sequence will increase as many times as the number of slices and could be nearly 20 times longer in this case. Lastly, since we would need to use proton density and T_2^* weighting with a long TR these images acquired with longer duration may suffer from large motion artifacts as well.

Compared to the standard Gd-based perfusion angiographic technique, as shown in Figure 6, BOLDangio appeared to express higher specificity for vasculature imaging, because the Gd-based angiograms had a lower contrast to noise ratio as compared to BOLDangio. This difference may be due to the short acquisition window required to image the fast perfusion process. Hence gadolinium-based angiography may be highly dependent on injection methods (dose or rate), acquisition timing, potential leak/deposit of Gd into tumor tissue with its compromised blood brain barrier. To solve this problem, fast imaging techniques and comprehensive mathematic models have been proposed for dynamic contrast enhanced MRI (DCE-MRI) (55–57).

To improve the validity of our findings even more, immunohistological staining of vasculature using for example Von Willebrand factor (vWF) antibodies (9) might help us to correlate our experimentally determined BOLDangio contrast with histologically determined measures of vascular density. Unfortunately, the slice thickness required to perform histology is about 5–10 μm , a small fraction of the MRI imaging slice thickness (1/50 to 1/100 as thick). Therefore, our experimental values would be quite different from anything arrived at histologically. Additionally, histological quantification of a brain volume is very time consuming and subject to sampling errors. As it is well known that BOLD contrast is purely from blood vessels, vessel staining using histology technique may not be that critical.

Conclusions

Blood Oxygen Level Dependent Angiography (BOLDangio) has proven to be a verifiable alternative technique for vasculature imaging and has the potential to become a powerful tool for pre-clinical research. The ability to collect high resolution normal and tumor vasculature angiographs with BOLDangio that can non-invasively detect tumor metastasis, associated hemorrhage, and hematoma has been sufficiently demonstrated and suggests that BOLDangio may be used to monitor the effects of anti-angiogenic drugs and therapies. Furthermore, since BOLDangio contrast originates from blood in the circulation, its potential applications may even extend to stroke and Alzheimer's disease associated vasculature abnormalities (58), subcutaneous tumors, as well as cardiovascular atherosclerosis induced angiogenesis.

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Abbreviations

BOLD	blood oxygen level dependent
BOLDangio	blood oxygen level dependent angiography
dHb	deoxyhemoglobin
Gd	gadolinium
GRE	gradient echo
ROI	region of interest
SO₂	oxygen saturation
TOF	Time of flight
MIP	maximum intensity projection

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Figure 1.

(A–C) T_2^* -weighted MRI images of a representative normal brain slice at different TE (5, 10, 15, 20 ms) acquired under 100% O_2 (A), the corresponding R_2^* map (B) and images at different oxygen inhalations (C from left to right: hyperoxia 100% O_2 , normoxia 21% O_2 , hypoxia 15% and 8% O_2) at 10ms TE. Red arrows indicated typical ROIs of vessels. D. BOLD contrast maps calculated with 100%, 15% and 8% of oxygen compared to normoxia inhalation respectively. E. Oxygen saturation (SO_2) curves of arterial and venous blood as a function of inhalation oxygen.

Figure 2.

A. R_2^* relaxation rate of normal brain tissue (mostly gray matter) and vessels as a function of inhalation oxygen. B. Calculated BOLD contrast (TE 10ms) with hyperoxia (100% O_2) or hypoxia (15% and 8% O_2) compared to normoxia (21% O_2) inhalation. Significant difference of BOLD contrast between vessel and brain tissue is observed under 8% O_2 (* $p < 0.05$). C. Calculated TE dependence of BOLD contrast under hypoxia inhalation of 8% O_2 .

Figure 3.

BOLDangio imaging of a tumor rat. GRE images under normoxia, 15% of Hypoxia and 8% of Hypoxia (A–C). BOLD maps at 8% or 15% O₂ are shown from D and F respectively. Histograms of BOLD effect at 8% (E) and 15% O₂ (G). Tumor vasculature is shown as ring-shaped structure at 8% of BOLD contrast map.

Figure 4.

Demonstration of BOLDangio MRI for detecting tumor metastasis and hemorrhage. GRE images under normoxia and hypoxia (A–B). Tumor hemorrhage sites are labeled with red arrows in A. BOLD contrast map is color-mapped in C or shown in gray scale (D). Metastatic tumors (yellow and green circles) are derived from the edge of the primary tumor (red circle), where the tumor cells were initially implanted.

Control Brain

Tumor Brain

Figure 5. MIP images of a normal (A) and tumor (B) brains clearly shows normal and tumor vasculature.

Figure 6. (A–D) T2* weighted GRE images of the same slice under normoxia (A), 8% of Hypoxia (B), the corresponding BOLD contrast map (C) and BOLDangio MIP image of the tumor brain (D). (E–H) T1 weighted GRE images of the same slice before (E), during Gd-DTPA perfusion (F), corresponding contrast map due to Gd (G) and multi-slice MIP image (H).