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Water exchange-minimizing DCE-MRI protocol to detect changes in tumor vascular parameters: effect of bevacizumab/paclitaxel combination therapy

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Abstract

Objective—The purpose of this study was to assess changes in the tumor microvasculature induced by combination antiangiogenic therapy in MCF-7 breast tumor mouse models, using a noninvasive DCE-MRI method that minimizes the effect of water exchange.

Materials and methods—3D quantitative DCE-MRI images were acquired with a heavily T_1 -weighted saturation recovery gradient echo sequence with a recovery delay of 20 ms. Tumor vascular volume (VV) and vascular permeability-surface area product (PS) were obtained through a linear regression of the albumin-Gd-DTPA-enhanced dynamic image intensity on MCF-7 breast tumor mouse models treated with combination bevacizumab/paclitaxel therapy.

Results—Measured tumor VV values were significantly higher than the values that have been reported previously using quantitative T_1 mapping, and are in good agreement with micro-CT (computed tomography) results reported earlier from other tumor models. A trend of decreasing tumor PS was detected in the group of MCF-7 tumor bearing mice treated with the bevacizumab/paclitaxel combination regimen.

Conclusion—VV and PS maps obtained by a heavily T_1 -weighted acquisition protocol revealed the large peripheral blood vessels as well as the permeable areas within the tumor. A 12-day/three-dose combination treatment of bevacizumab and paclitaxel resulted in delayed tumor growth and a trend of decreasing tumor vascular permeability surface area product.

Keywords

DCE-MRI; Water exchange; Tumor vascular volume and vascular permeability; Combination antiangiogenic therapy

Introduction

DCE-MRI has been extensively used in pre-clinical and clinical trials to evaluate the efficacy of antiangiogenic therapy. Functional tumor microvascular parameters, such as

vascular volume and vascular permeability, are derived from the pharmacokinetic modeling of the T_1 -weighted DCE-MRI time-course data and used as surrogate efficacy biomarkers [1, 2]. True quantitative DCE-MRI (qDCE-MRI) requires the quantitative measurement of the image intensity as well as the quantitative analysis of the dynamic data. Multiple challenges still remain in the quest to standardize the DCE-MRI technique in order to produce reliable and reproducible quantitative biomarkers [3]. These challenges include how to acquire the quantitative T_1 -weighted MRI signal, how to derive the contrast concentration from the MRI signal, and finally, how to analyze the time-dependent DCE-MRI image intensity. While the majority of the current discussions has been focused on the quantitative analysis of the raw DCE-MRI image data, clear recommendations regarding the optimal quantitative imaging acquisition protocol are still lacking [1].

An inherent issue for the quantitative analysis of the DCE-MRI data is that the measured intensity of the T_1 -weighted MRI signal is generally not directly proportional to the concentration of the contrast agent (CA) in the tissue [4, 5]. Subsequently, CA concentration is derived from the enhanced T_1 relaxation rate, which is calculated from a post-CA T_1 -weighted MRI intensity and the baseline T_1 relaxation rate [6], but complications can arise when CA distribution within a single imaging voxel is not uniform in biological tissues. Such a heterogeneous imaging voxel is composed of compartments of different T_1 relaxation rates if the intercompartment water exchange is limited. The T_1 relaxation recovery of these voxels follows a multi-exponential function so that it cannot be represented by a single T_1 relaxation rate. The practice of using one T_1 relaxation rate for the entire image voxel effectively has assumed a fast water exchange between all compartments within the voxel, and has ignored any possible nonuniform CA distribution. Therefore, CA concentration calculated from such T_1 relaxation rate will vary as the imaging parameters change and will not always be accurate [7].

In principle, the effect of the water exchange can be minimized and the accuracy of the CA concentration determination can be improved by increasing the T_1 weighting of the DCE-MRI imaging protocol with a large flip angle and short recovery time [7]. A heavily T_1 -weighted acquisition produces enhanced MRI signals that are linearly proportional to the CA concentration, so that an average voxel CA concentration can be determined from the enhanced MRI signal intensity, regardless of the intercompartment water exchange kinetics [7]. The downside of a heavily T_1 -weighted protocol is its low signal-to-noise ratio, which may reduce the quality of the pharmacokinetic fitting of time-course data [8]. A remedy for the low signal-to-noise ratio of a heavily T_1 -weighted acquisition is to increase the number of acquisition averages, but this is at the expense of decreased temporal resolution as the acquisition time becomes longer. Additionally, it may fail to record the initial steep enhancement slope in the lesion following the administration of a low molecular weight CA, such as Gd-DTPA, which has a short circulation half-life and extravasates rapidly. However, a lower temporal resolution may be adequate for the dynamic MR imaging with a macromolecular contrast agent (MMCA) that maintains a steady vascular concentration and leaks slowly from the blood vessels.

The addition of the humanized anti-VEGF monoclonal antibody, bevacizumab, to breast cancer treatment has become a hotly debated issue recently [9, 10]. While early results

indicated that bevacizumab in combination with taxane chemotherapy improved the progression-free survival rate [11], later reports have shown virtually no difference in pathologic complete response rate in patients who received neoadjuvant chemotherapy with and without bevacizumab [12]. However, a noninvasive quantitative study of the vascular changes in tumors induced by bevacizumab/paclitaxel combination therapy is still lacking.

Based on the above considerations, we used a water exchange-minimizing, heavily T_1 -weighted qDCE-MRI technique with an MMCA, albumin-Gd-DTPA for the quantitative determination of tumor vascular parameters during the course of bevacizumab/paclitaxel combination therapy in mice bearing human breast cancer MCF-7 tumors. 3D qDCE-MRI images were acquired with a heavily T_1 -weighted short saturation recovery gradient echo protocol. The concentration of albumin-Gd-DTPA was derived directly from this heavily T_1 -weighted image intensity instead of the T_1 maps as reported earlier [2, 13]. Tumor vascular parameters before and after the bevacizumab/paclitaxel treatment were compared, and mice treated with saline were used as controls.

Materials and methods

Human breast cancer xenograft in mice

Severe combined immunodeficient (SCID) mice (female, 4–6 weeks old, approximately 22 g body weight) were purchased from the NCI (Bethesda, MD, USA). Mice were maintained on sterilized food and water. The human breast carcinoma cell line, MCF-7, was originally obtained from ATCC (Manassas, VA, USA.) and maintained in Eagle's minimum essential medium (EMEM), with 1 % penicillin/streptomycin and 10 % fetal bovine serum at 37° C with 5 % CO₂. Pellets of 17 β -estradiol (0.18 mg, 60 day release) were obtained from Innovative Research of America (Sarasota, FL). Breast tumors in SCID mice were established by inoculating 3×10^6 MCF-7 cells (in 0.05 ml of Hanks' balanced salt solution) into the thoracic mammary fat pad 48 h after the implantation of one half of a 17 β -estradiol pellet in the back of the mouse. All animal experiments in this study were performed in accordance with the Guidelines for Animal Experimentation of the Johns Hopkins School of Medicine.

Treatment and scanning schedule

Combination therapy with antiangiogenic bevacizumab and cytotoxic paclitaxel was initiated after tumors reached approximately 150 mm³ in volume. MCF-7 breast tumor xenograft-bearing mice were treated with three cycles of bevacizumab and paclitaxel by intraperitoneal injection, each at 10 mg/kg every four days, doses comparable to those reported earlier in breast tumor-bearing mice [14]. qDCE-MRI scanning was performed prior to the treatment and on day 12 after the initiation of the treatment. Mice in the control group were treated with equal volumes of saline and were subjected to the same scanning protocol. Tumor volumes were determined from the length of the longest axis and the width of the vertical axis, measured with a slide caliper, as described previously [15]. Overall, 14 mice were scanned prior to the treatment, with six mice scanned at day 12 for the control and combination treatment groups, respectively.

MRI data acquisition and analysis

All MRI studies were performed on a horizontal 30-cm bore 9.4T Bruker Biospec preclinical scanner with a maximum gradient strength of 400 mT/m. Anesthesia was initiated with 4 % isoflurane and maintained with a 2 % isoflurane/oxygen mixture. The tail vein of mice bearing MCF-7 xenografts was cannulated for the injection of the MMCA albumin-Gd-DTPA prior to each scanning. Anesthetized mice were placed in a plastic cradle with the tumor positioned in a custom-built, single-turn volume coil positioned orthogonal to the B_0 magnetic field. Albumin-Gd-DTPA was prepared in our laboratory as described previously [13, 16]. This agent has high stability and minimal toxicity in vivo when used at doses of up to 0.6 g/kg, as demonstrated by many years of application in research [13, 17].

A proton density (PD)-weighted image was acquired prior to the administration of the contrast, with a 3D gradient echo sequence, TE/TR = 1.5/8 ms and a nominal flip angle of 5° . A quantitative M_0 map was derived from this PD-weighted image, using a calibration constant determined from two gradient echoes acquired separately at the center of the 3D k -space without phase-encoding gradients: TE/TR = 1.5/10,000 ms and a 90° flip angle; and TE/TR = 1.5/8 ms and a nominal 5° flip angle. All above scans were acquired with 10 averages.

Heavily T_1 -weighted images were acquired using a saturation-recovery 3D gradient echo sequence with the following elements: a magnetization saturation block consisting of a composite 250 μ s length, 90° ($90_0 180_{120}$) RF pulse [18], followed by variable crusher gradients and a short recovery time, TD, of 20 ms; a 250 μ s length, 90° slab-selecting readout pulse; and a gradient echo with TE = 1.5 ms (Fig. 1). The total duration of this acquisition sequence was 27 ms, resulting in a total acquisition time for a 3D image set of about six minutes, with an FOV of $12 \times 12 \times 10$ mm, a matrix size of $128 \times 64 \times 48$, and six averages. A baseline image was collected before the intravenous administration of albumin-Gd-DTPA at 0.16 mmol (Gd)/kg, and dynamic contrast-enhanced images were collected for 30 min after the injection. At the end of the dynamic imaging studies, the mouse was removed from the magnet and a sample of blood was taken from the tail vein ($\sim 20 \mu$ l). T_1 of this blood was measured using an inversion recovery technique, with relaxation delays in the range of 25 ms–6.4 s. The measured post-CA blood T_1 value and a pre-CA blood T_1 value of 1.5 s were used to calculate the relaxivity of albumin-Gd-DTPA [19]. Data analysis was performed using in-house programs developed in the IDL language (Exelis Visual Information Solutions, Inc., Boulder, CO) that provided 3D sine apodization, zero filling to $128 \times 128 \times 128$, and magnitude Fourier transformation of raw data, as well as kinetic analysis of the dynamic image data. Images were visualized using the ImageJ (National Institutes of Health, Bethesda, MD) and Amira (Visage Imaging Inc., Richmond, VIC, Australia) packages.

Modeling CA pharmacokinetics from qDCE-MRI data

The signal intensity M of a gradient echo is described as

$$M = M_0 \cdot \frac{\sin \alpha (1 - e^{-TR/T_1})}{1 - \cos \alpha \cdot e^{-TR/T_1}} \quad (1)$$

where α is the flip angle, TR is the repetition time, and M_0 is a constant related to the proton density as well as the experimental conditions. T_2 effect was considered negligible in our study due to the short TE used. We used the same number of averages for all acquisitions in the following equations for simplicity.

A 3D proton density map M'_0 was acquired with a gradient echo sequence, TE/TR = 1.5/8 ms, and a nominal flip angle of 5° . The quantitative M_0 map was subsequently derived from this proton density map calibrated by the intensity of two gradient echoes acquired separately at the center of the 3D k -space without any phase-encoding steps: E_1 of the fully relaxed echo with TE/TR = 1.5/10,000 ms and a 90° flip angle; and E_2 of the echo at the same conditions of M'_0 at TE/TR = 1.5/8 ms, and 5° flip angle as

$$M_0 = M'_0 \cdot \frac{E_1}{E_2} \quad (2)$$

T_1 -weighted qDCE-MRI signals were acquired with a short saturation delay (TD) recovery gradient echo method with a 90° read pulse. The intensity of this T_1 weighted signal is described as:

$$M = M_0 \cdot \left(1 - e^{-\frac{TD}{T_1}} \right) \quad (3)$$

Under a heavily T_1 weighted condition with a very short saturation recover delay $TD \ll T_1$, Eq. (3) can be simplified to:

$$M = M_0 \cdot \frac{TD}{T_1} \quad (4)$$

The tissue CA concentration, C_t is calculated from the pre-and post-CA T_1 relaxation time $T_{1 \text{ pre}}$ and $T_{1 \text{ post}}$ as:

$$C_t = \frac{1}{R_1} \cdot \left(\frac{1}{T_{1 \text{ post}}} - \frac{1}{T_{1 \text{ pre}}} \right) \quad (5)$$

where R_1 is the T_1 relaxivity of albumin-Gd-DTPA in the interstitium. Combining Eqs. (4) and (5), C_t can be derived directly from the pre- and post- T_1 -weighted image intensity, as well as M_0 :

$$C_t = \frac{M_{\text{post}} - M_{\text{pre}}}{R_1 \cdot TD \cdot M_0} = \frac{\Delta M}{R_1 \cdot TD \cdot M_0} \quad (6)$$

Assuming that the relaxivity of albumin-Gd-DTPA is the same in the blood and the interstitium [20], the CA concentration in the blood C_b can be derived from the T_1 relaxation time changes as:

$$C_b = \frac{1}{R_1} \cdot \left(\frac{1}{T_{1\text{post}}^b} - \frac{1}{T_{1\text{pre}}^b} \right) = \frac{1}{R_1} \cdot \Delta \left(\frac{1}{T_1^b} \right), \quad (7)$$

where $T_{1\text{pre}}^b$ and $T_{1\text{post}}^b$ are the blood T_1 relaxation time before and after the injection of the albumin-Gd-DTPA, respectively. CA concentration in the plasma C_p and whole blood C_b is related through the hematocrit fraction Hct as:

$$C_b = C_p \cdot (1 - \text{Hct}). \quad (8)$$

Both C_b and C_p can be considered a constant during the entire DCE-MRI acquisition due to the ~4 h blood half-life of albumin-Gd-DTPA [21]. Combining Eqs. (6) and (7), the ratio of tissue CA concentration C_t against blood CA concentration C_b can be directly calculated from the T_1 -weighted signal enhancement M , the relaxation rate change in the blood, and M_0 :

$$\frac{C_t}{C_b} = \frac{\Delta M}{\text{TD} \cdot M_0} \cdot \frac{1}{\Delta \left(\frac{1}{T_1^b} \right)}. \quad (9)$$

The Patlak model was used to model the pharmacokinetics of albumin-Gd-DTPA [22, 23]. This model assumes that the efflux of the contrast agent from the interstitium back to blood vessels is negligible and a linear kinetic model can be used to derive vascular volume (VV in %) and permeability surface area product (PS, in ml/(g·min)):

$$C_t(t) = \text{VV} \cdot (1 - \text{Hct}) \cdot C_p(t) + \text{PS} \cdot \rho \int_0^t C_p(\tau) d\tau, \quad (10)$$

where ρ is the tissue density, which is commonly assumed to be 1 g/ml [24]. Combination Eqs. (8) and (10):

$$\frac{C_t(t)}{C_b} = \text{VV} + \frac{\text{PS}}{1 - \text{Hct}} \cdot \rho \cdot t. \quad (11)$$

Thus the 3D voxel-by-voxel VV and PS/(1-Hct) parametric maps of MMCA were derived from the intersect and slope of the linear regression of the ratio $\frac{C_t(t)}{C_b}$, calculated from Eq. (9), versus elapsed time after the administration of albumin-Gd-DTPA, respectively, as described previously [25, 26]. PS was calculated from the above slope using a Hct value of 0.4 [27]. A total of five time points over a period of 30 min was used in the linear regression. The blood T_1 was only determined at the end of the each qDCE-MRI time-series acquisition and $\Delta \left(\frac{1}{T_1^b} \right)$ was calculated using a pre-contrast T_1^b value of 1.5 s. Only positive

intercepts and slopes were retained as physically relevant VV and PS values and the VV and PS values of the voxels with negative intercepts or slopes were set to zero in the VV and PS parametric map. However, these voxels with zero VV or PS values were included in the calculation of the mean tumor VV and PS through the voxel counts.

Statistical analysis

The non-parametric Kruskal–Wallis test was used to compare the tumor volume, mean VV, and mean PS values of the tumors of different groups, using StatPlus®.mac (AnalystSoft Inc., Alexandria, VA) software. Paired analysis was not used since the mice scanned on day 0 and day 12 were not always the same. Mean parameters from each group were compared between groups (pre-treatment, post-treatment on day 12, and control on day 12).

Results

qDCE-MRI experiments were performed with a control group ($n = 6$) and treatment group ($n = 6$) of mice. Representative images of tumors scanned pre-treatment and 12 days post-treatment are shown in Fig. 2. The pixel-by-pixel difference images (Fig. 2 column c) show that visible albumin-Gd-DTPA enhancement is mostly limited to the tumor periphery immediately after administration. The enhancement slowly spread to the tumor interior over the duration of the qDCE-MRI acquisition (Fig. 2 column d). Consistent with the enhanced permeability and retention property of the tumor [28], enhancement of the albumin-Gd-DTPA lingered and was observed in the tumor core up to 12 days after the injection (Fig. 2, images 2b, 3b). However, signals from the CA accumulated from previous injection were absent on the difference images acquired after the current CA injection, as demonstrated in Fig. 2, images 2c and 3c. Figure 3 shows a typical enhancement image observed in mice in all three groups, as well as the linear regression of $C_t(t)/C_b$ as a function of time of three regions of interest (ROI). The enhancement in the blood vessel (blue ROI) appeared immediately in the first frame acquired after the administration of the albumin-Gd-DTPA and remained stable over the time of the experiment. An area near the tumor rim (green ROI) showed moderate increase in enhancement, while the enhancement in a core area (red ROI) was minimal during the entire DCE-MRI duration. Linear regressions of the CA concentration normalized by the blood CA concentration of these ROIs are shown in Fig. 3, panel b. VV and PS values were obtained from the intercept and slope (corrected for hematocrit fraction) of the linear regression, respectively. The vessel area (blue) had a VV value of ~100 % and zero permeability, the area near the rim (green) had moderate VV and PS values, and the core area (red) had minimal VV and PS values. A 3D VV parametric map and the corresponding MR angiography-type image of the enhanced tumor blood vessels acquired at the first time point after the injection of albumin-Gd-DTPA are shown in Fig. 4. It illustrated that the reconstructed VV map included both contribution from the macroscopic blood vessels and the leaky microvasculature. VV and PS maps of a central slice of a tumor are shown in Fig. 5. Although higher VV and PS were both observed near the tumor rim, the superimposed image of VV and PS (Fig. 5c) demonstrated that there was little spatial correlation between tumor regions of high vascular volume and high vascular permeability, even though PS, the product of vascular permeability and vascular surface area, is related to the vascular volume. The overall mean correlation between VV and PS

maps for all mice in the control and treated groups was 0.14 and 0.11, respectively. A scatter plot of PS versus VV from a representative tumor is shown in Fig. 6, which also demonstrated that tumor areas with high VV values were generally less permeable.

For the calculation of the mean values of VV and PS, a 3D region of interest was drawn around the tumor slice-by-slice. Changes in mean tumor volume, VV and PS following the treatments are summarized in Fig. 7. Measurements of VV and PS from day 0 for the treatment and control groups were combined as the pre-treatment group. Statistical analysis showed that the combination treatment induced a significant delay in tumor growth compared to the control group ($p = 0.02$). However, the change in PS in the treatment group was not significant ($p = 0.11$) relative to the control or the pre-treatment group, although a downward trend was observed as opposed to the control group. There was no significant change in vascular volumes ($p = 0.71$) between both the treatment and controlled groups.

Discussion

Our goal in this study was to acquire qDCE-MRI data using albumin-Gd-DTPA with a heavily T_1 -weighted method to reduce sensitivity to water exchange kinetics, and to obtain accurate values of tumor vascular parameters. These vascular parameters were used to evaluate the combination therapy with bevacizumab and paclitaxel on the human breast cancer MCF-7 mouse model. A previously used method, based on a 2D saturation-recovery T_1 measurements, with recovery delays of 100, 500, 1,000, and 7000 ms, and a flip angle of 10° s in the same tumor model, produced significantly lower average VV values of 0.3–1 % [13, 17], relative to the current VV values around 6 % (Fig. 6) prior to any treatments. This discrepancy in VV, which can be caused partially by differences in population, is compatible with the six-fold difference in tumor blood volume calculated based on the no-exchange and fast-exchange models reported by Donahue et al. [7]. However, the PS values measured by both methods are comparable.

Modeling PS and VV values based on CA concentration derived from T_1 maps invariably used a single T_1 relaxation rate within an imaging voxel, which effectively assumed fast water exchange between compartments of different CA distribution. When an MMCA, such as albumin-Gd-DTPA, is administered, plasma protons can have a T_1 relaxation time below 200 ms, while the extravascular compartment has a much longer T_1 of about 1–2 s at 9.4 T. Consequently, imaging voxels comprised of both intravascular and extra-vascular compartments with a large T_1 difference are no longer in the fast-exchange limit [6]. To assume fast-exchange under this situation can lead to serious underestimation of the tumor VV, especially when long relaxation delays are used to measure the T_1 maps [7]. The fast relaxing components such as blood vessels within an image voxel becomes underestimated when the relaxation delays used to generate the T_1 maps are comparable to the blood vessel T_1 relaxation time. Such long relaxation delays are not adequate to sample the fast relaxing components [20]. Therefore, the main contribution to the resultant apparent T_1 maps is from the slow T_1 relaxing components [20]. The short saturation recovery method with a relaxation delay, TD, of 20 ms used in the current study produces signal intensities that are linearly dependent on the T_1 relaxation rates for both intravascular compartment with high CA concentrations and for the extravascular compartment with significantly lower CA

concentrations at $TD \ll T_1$ [7]. As can be seen in Fig. 2 and Fig. 3, the enhanced macroscopic vasculature is clearly visible with the current technique. The calculated VV map (Fig. 4) shows the inclusion of these vessels as well as the permeable microvasculature that cannot be resolved in MR images. The significant difference in the average tumor vascular volume between the current and previous methods is most likely due to the contribution to the total VV from the steadily enhanced macroscopic vasculature, which was inconspicuous in the VV maps derived from images acquired earlier with long relaxation delays.

However, the T_1 mapping and the current qDCE-MRI method produced similar PS values, most likely due to the low permeability of albumin-Gd-DTPA in the current tumor model. Figures 2 and 3 showed the relatively weak enhancement beyond the macroscopic blood vessels, where the tumor tissue is more likely to be permeable. The fast-exchange limit may be a good approximation in such areas with weak enhancement as the T_1 variation is small across interstitium and intracellular compartments such that the CA concentration derived from the current method and the T_1 mapping are comparable [6].

Our qDCE-MRI results showed the MCF-7 tumor vasculature is highly heterogeneous. Figure 3 demonstrates that stably-enhanced tumor blood vessels showed practically a 100 % VV and zero permeability, which supports the early findings that highly vascularized tumor areas are less leaky and have significantly lower permeability to albumin-size compounds [13]. Additionally, the scatter plot in Fig. 6 indicates that areas with high VV are generally associated with low PS. Previous DCE-MRI study with Gd-DTPA also showed a permeable tumor rim and less perfused tumor core with few small necrotic areas in the untreated MCF-7 tumor [29]. Figure 6 illustrates that a dominant portion of the tumor showed both low VV and PS, which is consistent with the relative low blood vessel profile density of the MCF-7 tumors [30] and distribution patterns of albumin-Gd-DTPA reported for this tumor model [31].

In addition to the highly T_1 -weighted qDCE-MRI acquisition, we have also developed a method to acquire M_0 map in a short time. By calibrating the proton density map acquired at a short TR with two gradient echoes acquired separately at the center of the 3D k -space without any phase-encoding steps, we have bypassed the need to acquire the full matrix with a complete T_1 relaxation recovery. The accuracy of this method is supported by the measured VV values close to 100 % for the macroscopic tumor blood vessels, which also can be served as an internal reference. Although the MMCA used in this report is not clinically translatable due to its immunogenicity, the current M_0 map acquisition method can be adapted in the clinical setting to provide an alternative to the commonly used variable flip angle method [32]. Our results also suggested that a heavy 3D T_1 -weighted protocol (large flip angle and short TR) should be helpful to acquire the dynamic data while maintaining a fine balance between T_1 -weighting and the measured signal amplitude.

Our current results are in close agreement with results reported for human cancer rat experimental models, also obtained with albumin-Gd-DTPA with a heavily T_1 -weighted gradient echo sequence, with TE/TR = 1.4/50 ms and a 90° flip angle [2, 33]. Sennino et al. [34] have reported a fractional plasma volume of the RIP-Tag2 tumor at 2.4–14.1 %, also

measured by albumin-Gd-DTPA DCE-MRI that is consistent with our values. Mouse Lewis lung carcinoma was shown to have a vascular volume around 5 %, determined by high resolution micro-CT (computed tomography) after in vivo perfusion with Microfil® [35]. MDA-MB-231 mouse tumor xenografts were reported to have a vascular volume of ~5 %, measured by micro-CT [36]. It should not be a surprise that DCE-MRI produced higher vascular volumes, since it includes the vascular volume of capillaries (corrected for permeability) that are not rendered by Microfil®CT due to the limited efficiency of filling in the capillaries and the volume averaging effect during thresholding of the vasculature in micro-CT images [35]. A comparison of the 3D rendering of the VV map and the directly enhanced blood vessels on an early MR difference image depicts the additional vascular volume assayed by DCE-MRI, as illustrated in Fig. 4a, b.

When administered as a single agent, micro-CT measurements showed that a 14-day treatment with bevacizumab alone resulted in a reduction in both vascular volume and microvessel diameter of lung tumors in mice [35]. However, no significant tumor volume as well as microvascular changes were observed in an earlier albumin-Gd-DTPA DCE-MRI study in a rat MDA-MB-435 experimental tumor model following a nine-day treatment of bevacizumab [2], consistent with the largely negative therapeutic efficacy of bevacizumab monotherapy [37]. Although it was reported that the humanized antibody bevacizumab had limited reactivity towards the murine host VEGF [38], it appeared that anti-VEGF therapies could be effective against mouse models of human tumors [39]. Our investigation based on small groups of mice showed that a combination of three cycles of bevacizumab and paclitaxel within 12 days resulted in delayed tumor growth, as well as a trend towards reduction in vascular permeability in MCF-7 tumors. All these results reveal the significant complexity of the tumor vasculature and antiangiogenic interventions, as well as the importance of developing reliable noninvasive imaging markers for tumor response to therapy.

Despite the current controversy surrounding bevacizumab, it has been reported that its use, in combination with either paclitaxel or docetaxel, has been associated with prolonged progression-free survival and increased response rates in the first-line treatment of patients with metastatic breast cancer [40], especially for human epidermal growth factor receptor 2 (HER2)-negative locally recurrent or metastatic breast cancer [41]. The addition of bevacizumab to neoadjuvant chemotherapy significantly increased the rate of pathological complete response in triple-negative patients [42]. Our study showed that a combination treatment of MCF-7 tumors (HER2-low, estrogen and progesterone receptor positive), with three cycles of bevacizumab and paclitaxel within 12 days, resulted in delayed tumor growth, as well as a trend towards reduction in vascular permeability.

Conclusion

We have utilized a short saturation recovery time, highly T_1 -weighted qDCE-MRI imaging sequence that minimizes the effects of water exchange on the quantitation of contrast agent kinetics. We determined vascular parameters VV and PS of the HER2-low and estrogen and progesterone receptor-positive MCF-7 breast cancer model xenografted in mice before and after a bevacizumab/paclitaxel regimen, through the linear regression of the time series

qDCE-MRI intensities. A 12-day/three-dose combination treatment of bevacizumab and paclitaxel resulted in delayed tumor growth, as well as a trend of reducing tumor vascular permeability.

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Fig. 1.

A block diagram of the saturation-recovery T1-weighted imaging sequence that consists of a 90° composite magnetization saturation pulse, followed by a crusher gradient and a short recovery time, TD, of 20 ms; a 90° slab-selecting readout pulse; and a gradient echo with TE = 1.5 ms. The total duration of this acquisition is 27 ms

Fig. 2.

Representative **a** proton density-weighted; **b** corresponding pre-contrast T1-weighted; **c** one-minute; and **d** 25-minute postcontrast-enhanced T1-weighted images at the tumor center after the subtraction of the pre-contrast image. *Row 1*: pre-treatment; *Row 2*: day 12 of bevacizumab/paclitaxel; and *Row 3*: day 12 of saline control

Fig. 3.

a A representative enhanced image from a central slice of a tumor with three ROIs selected: blood vessel (*blue*), peripheral area (*green*), and core (*red*); **b** corresponding linear regression of $C_t(t)/C_b$ of the three selected ROIs, $C_t(t)/C_b$ are shown in different scale for blood vessel *blue circle* (left axis), and the peripheral *green triangle* and core *red square* (right axis) to reflect the magnitude difference

Fig. 4. Three-dimensional images reconstructed from the qDCE-MRI data. **a** 3D rendering of the enhanced vasculature immediately after the albumin-Gd-DTPA injection; **b** 3D calculated VV (%) map; **c** 3D calculated PS (ml/(g·min)) map

Fig. 5. Representative **a** VV (%) map; **b** PS (ml/(g-min)) map; and **c** overlay of PS and VV map of a central tumor slice

Fig. 6.
Scatter plot of PS versus VV from a representative tumor

Fig. 7.

Changes in **a** mean tumor volume of the saline control group (*crosses*) and the bevacizumab/paclitaxel treatment group (*shaded circles*) * $p < 0.05$, treatment versus control on day 12; **b** mean VV of the saline control group (*crosses*) and the bevacizumab/paclitaxel treatment group (*shaded circles*); and **c** mean PS of the saline control group (*crosses*) and the bevacizumab/paclitaxel treatment group (*shaded circles*)