Dynamic contrast-enhanced MR microscopy identifies regions of therapeutic response in a preclinical model of colorectal adenocarcinoma

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Purpose: A typical dynamic contrast-enhanced (DCE)-MRI study often compares the derived pharmacokinetic parameters on manually selected tumor regions or over the entire tumor volume. These measurements include domains where the interpretation of the biomarkers may be unclear (such as in necrotic areas). Here, the authors describe a technique for increasing the sensitivity and specificity of DCE-MRI by identifying tumor regions with a variable response to therapy.

Methods: Two cohorts ($n = 8/\text{group}$) of *nu/nu* mice with LS-174T implanted in the mammary fat pad were imaged at five time points over four weeks. The treatment/control group received bevacizumab/saline at a dose of 5 mg/kg or 5 ml/kg twice weekly; imaging experiments were performed weekly. MR images were acquired at an isotropic resolution of $156 \mu\text{m}^3$ (2.4 nl) and with a sampling rate of 9.9 s. The histogram of the time-to-peak (TTP) was used to identify two (fast- and slow-enhancing) regions based on a threshold of $\text{TTP} = 1000$ s. The regions were correlated with histology, and the effect of therapy was locally examined.

Results: Tumors in the treatment group had a significantly longer doubling time. The regions defined by thresholding the TTP histogram identified two distinct domains correlating significantly with tumor permeability and microvessel density. In the fast-enhancing region, the mean permeability constant (K^{trans}) was significantly lower in the treatment group at day 9; in the slow-enhancing region, K^{trans} was not different between the control and treatment groups. At day 9, the relative volume of the fast-enhancing region was significantly lower in the treatment group, while that of the slow-enhancing region was significantly higher.

Conclusions: Two regions with distinct kinetic parameters were identified based on the histogram of TTP. The effect of bevacizumab, as measured by a decrease in K^{trans} , was confined to one of these regions. High spatiotemporal resolution MR studies may contribute unique insights into the response of the tumor microenvironment to therapy. © 2015 American Association of Physicists in Medicine. [<http://dx.doi.org/10.1118/1.4917525>]

Key words: MRI, functional imaging, biological target volume

1. INTRODUCTION

Tumor neovasculature, often identified by leakiness, heterogeneous blood flow, and abnormal vessel diameter, is structurally and functionally different from healthy vessels.¹ Dynamic contrast-enhanced (DCE)-MRI has been widely used to study transfer kinetics between the intra- and the extravascular spaces, providing a set of *in vivo* biomarkers useful in diagnosis and assessment of response to therapy.² The analysis of the kinetic curves measured with DCE-MRI has traditionally been part of one of three frameworks: qualitative, semiquantitative, and quantitative. The qualitative and semiquantitative approaches rely on the hypothesis that the temporal evolution of the MR signal intensity is indicative of tumor viability.^{3–5}

Under these conditions, the measurement of the preinjection $T1$ map, needed in the calculation of the molar concentration of the contrast agent, is not necessary. Conversely, the quantitative approach is based on the analysis of the temporal evolution of the concentration of the contrast agent and in principle is independent of the acquisition hardware (MRI scanner, field gradients, RF coils) and imaging protocol. The biomarkers from DCE-MRI have been applied extensively in monitoring therapeutic response in solid tumors in the preclinical⁶ and clinical² domains.

The majority of studies measuring the effect of an antiangiogenic agent have compared the mean value of the derived biomarkers on manually selected regions of interest (ROIs) or over the entire tumor volume. These measurements

FIG. 1. Two cohorts ($n = 8/\text{group}$) of *nu/nu* mice with LS-174T implanted in the mammary fat pad were imaged at five time points over the course of four weeks. A baseline scan was acquired at day 0. Treatment (Tx) was first administered at day 1 at a dose of 5 mg/kg; the control group received normal saline at a dose of 5 ml/kg. The first post-treatment imaging experiment was performed at day 2. Thereafter, treatment/placebo was administered at the same respective dose twice weekly; imaging experiments were performed weekly. At completion of study, mice were perfusion-fixed for histology (CD31 and HE staining).

include regions in the tumor where the interpretation of the kinetic parameters is unclear (such as in necrotic areas) or where response to therapy may be heterogeneous.⁷ Several research groups, recognizing the extensive heterogeneity of the uptake parameters in the tumor volume, have attempted to focus the analysis at specific tumor locations.^{8–10} However, the DCE-MRI protocols employed in a typical preclinical experiment have been limited in spatial and temporal resolutions and/or coverage.¹¹ High spatiotemporal resolution acquisitions, which we refer to as dynamic contrast-enhanced-magnetic resonance microscopy (DCE-MRM), can reveal significant differences in kinetic parameters even at distances less than 500 μm .¹¹ We hypothesized that the incorporation of semiquantitative kinetic parameters in the analysis of DCE-MRM data may provide a way for identifying tumor subpopulations responding differently to therapy. This approach will be useful in cases when the calculation of fully quantitative kinetic parameters may not be possible.

In this work, we present findings from a longitudinal study in which the time-to-peak (TTP) parameter was used to automatically segment the tumor volume with the aim of examining the spatial distribution of therapeutic effect.

2. MATERIALS AND METHODS

2.A. Treatment protocol

All animal studies were approved by the Duke University Institutional Animal Care and Use Committee. Female *nu/nu* mice with a colorectal adenocarcinoma cell line (LS-174T, Charles River Laboratories, Wilmington, MA), implanted in the mammary fat pad, were followed longitudinally for four weeks. A diagram of the study design is shown in Fig. 1.

The animals were randomly assigned to a treatment ($n = 8$) or a control ($n = 8$) group, and a baseline scan was acquired at day 0. At day 1, bevacizumab (Avastin®, Genentech, San Francisco, CA) was administered via an intraperitoneal injection at a dose of 5 mg/kg; the control group received normal saline (0.9% sodium chloride) at a dose of 5 ml/kg. The first post-treatment imaging experiment was performed at day 2. Thereafter, bevacizumab/placebo was administered at the same respective dose twice weekly; imaging experiments were performed weekly.

At completion of the study, the mice were transcardially perfused to allow for comparison of histologic stains with DCE-MRM parameters. While anesthetized with Nembutal

FIG. 2. (a) K^{trans} map overlaid on contrast-enhanced MR image (colormap is in units of min^{-1}). (b) TTP map of same slice demonstrating the spatial distribution of region 1 ($0 \text{ s} \leq \text{TTP} \leq 1000 \text{ s}$) and region 2 ($1000 \text{ s} < \text{TTP} \leq \text{TTP}_{\text{max}}$). (c) Temporal evolution of contrast agent concentration in a single voxel at core and rim of tumor [arrows in panel (a)]. For ease of display, the number of temporal datapoints was reduced by a factor of 2. The lines show the smoothed curve calculated with the optimized Savitzky–Golay filter. (d) Histogram of TTP in tumor volume. Dashed line shows the threshold used to separate region 1 and region 2.

(Oak Pharmaceutical, Lake Forest, IL), saline was delivered transcatheterially for ~5 min at a flow rate of 8 ml/min followed by 10% buffered formalin for approximately the same time. The fixed specimens were immersed in a 10% formalin solution for 1 day. Given the location of the tumors in this study, whole-body perfusion/fixation was necessary for matching histology slides to MR imaging planes using anatomical landmarks. Four to six histology slides with a thickness of 5 μm were stained with CD31 or H&E in an alternating sequence. The brown channel intensity from a micrograph of the CD31 slide was used as a surrogate marker for microvessel density.¹²

2.B. DCE-MRM protocol

Measurements were acquired in a 7 T small animal MRI scanner (ParaVision 5.1, Bruker BioSpin MRI GmbH, Ettlingen, Germany) equipped with self-shielded gradient coils having a maximum strength of 450 mT/m and rise time of 110 μs . An actively detuned volume RF coil (linear transmit, ID = 72 mm) was used in conjunction with a four-element coil (2 $\times$ 2 linear array, 10 $\times$ 10 mm loops) for surface receive.

The time-dependent contrast agent concentration $C(t)$ was calculated using

$$C(t) = \frac{1}{r_1} (R1 - R1_0), \quad (1)$$

where $R1_0$ is the native relaxation rate and r_1 is the longitudinal relaxivity of the injected agent. The postinjection relaxation rate is found by^{13,14}

$$R1(t) = -\frac{1}{TR} \ln \left(\frac{1 - (A + B)}{1 - \cos(\alpha)(A + B)} \right),$$

$$A = \frac{S(t) - S(0)}{S_0 \sin(\alpha)}, \quad B = \frac{1 - E1_0}{1 - \cos(\alpha)E1_0}, \quad (2)$$

where $E1_0 = \exp(-TR * R1_0)$, α = flip angle, S_0 = proportionality constant including the equilibrium longitudinal magnetization and system gain, $S(0)$ = preinjection signal intensity, and $S(t)$ = time-dependent (postinjection) signal intensity.

In the framework of the extended Tofts model, the temporal evolution of the contrast agent concentration in the tumor is described by¹⁵

$$C(t) = v_p \cdot C_p(t) + K^{\text{trans}} \int_0^t C_p(u) \cdot e^{-(K^{\text{trans}}/v_e) \cdot (t-u)} du, \quad (3)$$

where $C_p(t)$ is the arterial input function (AIF), v_p is the fractional volume of the plasma compartment, v_e is the fractional volume of the extravascular-extracellular space (EES), and K^{trans} is the rate constant (measured in ml/min per ml of tissue) for the exchange of the agent across the microvascular endothelium. Equation (3) was solved on a voxel-by-voxel basis

FIG. 3. Correlation between (a) K^{trans} , (b) TTP regions, (c) CD31, and (d) H&E staining in a representative animal from the treatment group. (e) A significant correlation was found between K^{trans} and the intensity of the brown channel in a micrograph of a histology slide stained with CD31. The blue and red datapoints are from measurements in manually drawn ROIs in the core and rim of the tumor, respectively. (f) A comparison between region 1 and region 2 for all animals in this study shows that K^{trans} is significantly higher (approximately three-fold) in the fast-enhancing regions. TTP selects for regions with high permeability and microvascular density.

FIG. 4. TTP histogram [(a)–(d)], fast- and slow-enhancing regions [(e)–(h)], and K^{trans} map [(i)–(l)] as a function of tumor volume in a representative animal at day 0 [(a), (e), and (i)], day 2 [(b), (f), and (j)], day 9 [(c), (g), and (k)], and day 16 [(d), (h), and (l)]. Notice the change in the distribution of TTP as the tumor progresses. The K^{trans} and TTP maps reveal a clear correlation between high permeability values and fast-enhancing regions and low permeability values and slow-enhancing regions. Dashed line in panel (a) shows the threshold used to separate region 1 and region 2. Colormap is in units of min^{-1} .

using the linear least-squares method¹⁶ and the population AIF reported by Loveless *et al.*¹⁷

The pulse sequence and the k -space sampling strategy used in this study have been described elsewhere.¹¹ Briefly, an interleaved ultra-short-echo radial sampling sequence allowed for the reconstruction of 4D datasets using a sliding-window keyhole approach. The acquisition parameters were $\text{FOV} = 20 \text{ mm}^3$, $\text{matrix} = 128^3$, $\text{TR/TE} = 5/0.02 \text{ ms}$, $\text{NEX} = 1$, $\alpha = 10^\circ$, and 9.9 s temporal resolution. The native relaxation rate was measured with the variable flip angle technique¹⁸ using the same acquisition parameters and $\alpha = \{2^\circ, 10^\circ\}$.

Animals were positioned in a custom-made MR-cradle, maintained under anesthesia by isoflurane delivery via a nose cone, while body temperature was controlled between 36°C and 37°C by circulating warm water. Breathing was monitored through a pneumatic pillow, while adjusting isoflurane delivery to maintain a rate of 50–60 breaths/min. Dynamic imaging was initiated two minutes prior to contrast agent injection and lasted for approximately 20 min postinjection. An automatic syringe pump (KD Scientific, Inc., Holliston, MA) was used to administer Gd-DTPA (Magnevist, Schering AG, Berlin,

Germany) as a bolus via a 27-gauge tail vein catheter at a dose of 0.5 mmol/kg and flow rate of 2.4 ml/min .

2.C. Image analysis

We hypothesized that the analysis of the K^{trans} map based on the TTP map would enable the identification of tumor regions responding differently to antiangiogenic therapy. It is already known that TTP is inversely correlated to microvessel density.¹⁹ To calculate the TTP parameter, the signal intensity curves were smoothed with an optimized filter designed for the identification of signal amplitude.²⁰ Given a model for the noise power of the acquisition,²¹ we find that optimal smoothing is achieved with a single pass of a second order Savitzky–Golay filter with a window size of 15 points. Voxels where the peak enhancement was less than 10% of tumor peak enhancement were checked visually and assigned the maximum TTP if signal outliers biased the smoothing operation. This is justified because in these voxels the signal intensity is close to the noise floor. Once the maximum was estimated, the time to reach the peak of the signal was

calculated on a voxel-by-voxel basis. The TTP map was used to define two regions:

- Region 1 (fast enhancing): $0 \text{ s} \leq \text{TTP} \leq 1000 \text{ s}$.
- Region 2 (slow enhancing): $1000 \text{ s} < \text{TTP} \leq \text{TTP}_{\text{max}}$.

The tumor volume was manually segmented and the mask imported into the kinetic parameter maps obtained from DCE-MRM. The TTP parameter was used to generate two additional masks identifying the regions described above.

2.D. Statistical analysis

Mixed effect modeling was used to calculate tumor growth delay, tumor doubling time (DT), and to assess treatment effect on volume.²² The mean value of K^{trans} in the tumor volume, region 1, and region 2, was compared with the two-sample t -test. This test was also used to compare the relative volume of the regions defined from the TTP map. K^{trans} values outside the range $[0-1] \text{ min}^{-1}$ were omitted from the analysis. Significance was determined based on a P -level less than 0.05. The injection of the contrast agent in three datasets (out of 80) was unsuccessful. The K^{trans} values from those scans were not included in the analysis.

3. RESULTS

3.A. Biomarker correlation

Figure 2(a) shows a representative K^{trans} map overlaid on a contrast-enhanced $T1$ -weighted MR image. In Fig. 2(b), the K^{trans} map is compared to the TTP map of the same slice demonstrating in the spatial distribution of region 1 and in region 2. The temporal evolution of the contrast agent concentration in a single voxel at the core and rim of tumor is shown in Fig. 2(c). The fit lines reveal the effect of smoothing with the optimized Savitzky–Golay filter. The histogram of TTP in the entire tumor volume is depicted in Fig. 2(d), with the dashed line representing the threshold used to separate region 1 and region 2.

Figure 3 demonstrates the correlation between K^{trans} , TTP regions, CD31, and H&E staining in a representative specimen from the treatment group. A significant correlation was found between K^{trans} and the intensity of the brown channel in a micrograph of a histology slide stained with CD31. The blue and red datapoints are from measurements in manually drawn ROIs in the core and rim of the tumor, respectively (refer to Fig. S1 in the supplementary material³⁰ for details). A comparison between region 1 and region 2 for all animals in this study shows that K^{trans} is significantly higher in the fast-enhancing regions. The TTP parameter may be used to select for regions with both high permeability and microvascular density.

The evolution of the TTP histogram, the fast- and slow-enhancing regions, and K^{trans} map are shown in Fig. 4 for a representative animal at days 0, 2, 9, and 16. Notice the change in the distribution of TTP as the tumor progresses. Initially (days 0 and 2), as demonstrated by the relative volume of the fast-enhancing region, the tumor is highly perfused. At day 9,

FIG. 5. Tumor doubling time (DT) was significantly increased in the animals receiving bevacizumab. (a) Relative tumor volume ($\mu \pm \text{sem}$) as a function of time for the control and treatment arms. V_0 is calculated as the y -intercept of the volume vs time curve in a semilog scale. (b) Treatment extended the doubling time by more than two-fold (7.6 vs 16.1 days).

the TTP histogram and the distribution of region 1 and region 2 reveal a drastic change in overall enhancement. A comparison of K^{trans} and TTP maps emphasizes the correlation between high permeability values and fast-enhancing regions found in Fig. 3(f).

3.B. Identifying regions of therapeutic effect

The initial tumor volume ($\mu \pm \sigma$) for the treatment and control groups was $91 \pm 37 \mu\text{l}$ and $115 \pm 41 \mu\text{l}$, respectively. Figure 5 shows the effect of bevacizumab on tumor volume. The doubling time in the treatment arm was significantly longer than in the control arm. On average, treatment induced a tumor growth delay of approximately 8.4 days and extended the doubling time by more than two-fold.

The effect of bevacizumab on the permeability parameter and the relative volume of regions 1 and 2 are presented in Fig. 6. In the fast-enhancing region, the mean K^{trans} is significantly lower in the treatment group at day 9; in the slow-enhancing region, this parameter is not significantly different between the control and treatment cohorts at any point during therapy. At day 9, K^{trans} is significantly lower in the treatment group when the mean is taken over the entire tumor volume.

FIG. 6. Effect of bevacizumab on the [(a)–(c)] permeability parameter and [(d)–(e)] the relative volume of regions 1 and 2. (a) In the fast-enhancing region (region 1), the mean K^{trans} is significantly lower in the treatment group at day 9; (b) in the slow-enhancing region (region 2), this parameter is not significantly different between the control and treatment cohorts at any point during therapy. (c) At day 9, K^{trans} is significantly lower in the treatment group when the mean is taken over the entire tumor volume. (d) At the same time point, in the treatment group, the relative volume of region 1 is significantly lower, while (e) that of region 2 significantly higher. V_R = volume of region (1 or 2), V = tumor volume.

At the same time point, in the treatment group, the relative volume of region 1 was found to be significantly lower, while that of region 2 significantly higher than the respective region in the control group.

4. DISCUSSION

One of the main metrics for evaluating treatment response in solid tumors is lesion size.²³ Although a decrease in lesion volume, leading to a complete response, would be the most desirable outcome, the mechanism of action in some therapeutic agents may not cause complete tumor regression. Tumor growth studies that measure a treatment effect remain nonspecific in elucidating the purported mechanism of action. In the recent past, biomarkers from several imaging modalities have provided crucial complementary information to the findings of traditional clinical studies of anticancer therapies.^{24,25}

The noninvasive measurement of the spatial distribution of therapeutic effect can provide critical guidance into treatment planning. This study demonstrates the ability of dynamic contrast-enhanced magnetic resonance microscopy to identify tumor domains with a variable response to therapy. The radial-keyhole sampling technique used here allows for an increase by more than an order of magnitude in spatiotemporal resolution and coverage.¹¹ The approach can be readily translated to DCE-MRI in a clinical setting or to applications for imaging other tumor domains. For example, it has been demonstrated recently that hypoxic regions in a preclinical tumor model present with intermediate rates of contrast accumulation.²⁶ The ability to map hypoxia with high resolution DCE-MRI, combined with the soft tissue contrast inherent in MRI, would

aid in the definition of the biological target volume²⁷ and may have a profound impact in clinical care.

We show that a thresholding technique based on the histogram of the time-to-peak can pinpoint to subregions where the transfer constant is significantly reduced. The segmented subvolumes were located in predictable patterns, with the fast- and slow-enhancing regions in the periphery and core of the tumor, respectively. As shown in Fig. 3(f), the permeability parameter K^{trans} was significantly different in these domains. More importantly, CD31 and H&E staining reveal a similar spatial distribution of microvascular density, cell density, and DCE-MRM parameters. Previous work has also found a significant correlation between TTP and microvascular density.¹⁹ A separation of tumor volume into distinct regions derived from DCE-MRI has also been reported.⁹ Other investigators have attempted to use the signal intensity curves to derive biomarkers for discriminating between benign/malignant lesions²⁸ or as predictors for response to therapy.²⁹ Here, we find that the mean K^{trans} in the fast-enhancing region is significantly lower in the treatment group at day 9 of the regimen. The difference in this region determines the statistical difference in the mean of K^{trans} in the tumor volume, as there was no significant difference in the mean of K^{trans} in the slow-enhancing region. In this perspective, the fast-enhancing domains determine the true positive regions, while the slow-enhancing ones determine the true negative regions. If decrease in mean K^{trans} is assumed to indicate treatment success,² then the regions determined by the TTP parameter identify the tumor location where the therapeutic agent has its effect. At day 9, the fractional volume of the fast-enhancing region is significantly lower in the treatment group suggesting a direct therapeutic effect in regions of higher microvascular density.

The choice of the TTP threshold equal to 1000 s was empirical. The threshold was varied continuously until a match between the TTP regions, the K^{trans} map, and the histology slides was observed. Further analysis (Fig. S2 in the supplementary material³⁰) demonstrates the TTP parameter is estimated with a varying degree of precision as its value increases. Concentration curves were simulated as previously described.²¹ Following the smoothing operation with the Savitzky–Golay filter, it was found that larger TTP values were generally estimated with lower precision. In particular, the shape of the simulated TTP histogram when the maximum of the curve is at the last temporal point was found to be similar to the TTP histogram observed *in vivo*. The distribution of the parameter in this case justifies the empirical finding of a threshold approximate to 1000 s. Finally, the varying standard deviation of the estimated TTP distribution for different TTP values may be an explanation for the irregular shape of the distributions measured in this study.

5. CONCLUSION

The histogram of the time-to-peak provides useful knowledge about the spatial distribution of K^{trans} and microvascular density. Two regions with distinct kinetic parameters were identified when the TTP map from DCE-MRM was thresholded at 1000 s. The effect of bevacizumab, as measured by a decrease in K^{trans} , was confined to the regions which enhanced more rapidly. DCE-MRM studies may contribute unique insights into the response of the tumor microenvironment to therapy.

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³⁰See supplementary material at <http://dx.doi.org/10.1118/1.4917525> for histology/ K^{trans} images and simulation exploring the precision of TTP estimation.