Characterization of Tumor Angiogenesis With Dynamic Contrast-Enhanced MRI and Biodegradable Macromolecular Contrast Agents in Mice

Yi Feng,1,2 Eun-Kee Jeong,3 Aaron M. Mohs,1 Lyska Emerson,4 and Zheng-Rong Lu1*

The efficacy of polydisulfide-based biodegradable macromolecular contrast agents for characterizing tumor angiogenesis was investigated in a mouse model using dynamic contrast-enhanced MRI (DCE-MRI). Biodegradable macromolecular MRI contrast agents, gadopentetate dimeglumine (Gd-DTPA) cystamine copolymers (GDCC), and Gd-DTPA cystine copolymers (GDCP), with molecular weights of 20 and 70 kDa were used in the study. Gadodiamide (Gd [DTPA-BMA]) and albumin labeled with Gd-DTPA [albumin-(Gd-DTPA)] were used as the controls. The DCE-MRI studies were performed in nude mice bearing prostate tumor xenografts from the MDA-PCA-2b cell line. Tumor angiogenic kinetic parameters, including endothelial transfer coefficient (KPS), fractional tumor plasma volume (fPV), and permeability surface area product (PS), were estimated from the DCE-MRI data using a two-compartment model. The KPS and fPV values estimated by the biodegradable macromolecular contrast agents were between those estimated by Gd-DTPA-BMA and albumin-(Gd-DTPA). The parameters estimated by the agent with a slow degradation rate and high molecular weight, GDCP-70 (KPS = 2.09 ± 0.50 ml/min/100 cc and fPV = 0.075 ± 0.021), were closer to those by albumin-(Gd-DTPA) (KPS = 1.43 ± 0.64 ml/min/100 cc and fPV = 0.044 ± 0.007) than by other agents with relatively low molecular weight or rapid degradation rate. The polydisulfide-based biodegradable macromolecular contrast agents are promising for characterizing tumor vascularity and angiogenesis with DCE-MRI. Magn Reson Med 60:1347–1352, 2008. © 2008 Wiley-Liss, Inc.

Key words: DCE-MRI; tumor angiogenesis; biodegradable macromolecular contrast agent; Gd-DTPA cystamine copolymers; GDCC; Gd-DTPA cystine copolymers; GDCP

Tumor angiogenesis is essential for tumor growth and metastasis and the angiogenic tumor blood vessels are chaotic with high vascular permeability (1). Accurate assessment of tumor angiogenesis based on tumor vascular permeability or vascularity is crucial for tumor diagnosis and prognosis, assessment of tumor treatment efficacy, and cancer patient management (2–5). Dynamic contrast-enhanced MRI (DCE-MRI) can provide quantitative assessment of tumor vascular parameters such as the endothelial transfer coefficient (KPS), fractional tumor plasma volume (fPV), and permeability surface area product (PS) or related parameters (6,7). It has been shown that these quantitative parameters can be correlated to histological microvessel density and tumor angiogenesis (3). DCE-MRI can also be used to assess efficacy of tumor response to various therapies, including antiangiogenesis therapy (4).

Currently, DCE-MRI has been mostly performed by using low molecular weight Gd(III) chelates, e.g., gadopentetate dimeglumine (Gd-DTPA), as contrast agents to characterize tumor vascularity. These agents can be used to measure abnormal uptake of contrast agents in tumor tissue in comparison with the uptake in the surrounding normal tissue. However, they rapidly extravasate from the blood to the extracellular space in tumor tissue. Consequently, DCE-MRI with these low molecular contrast agents often gives overestimated results on tumor blood volume and vascular permeability (8). Therefore, it is difficult to accurately assess tumor angiogenesis with these agents (9,10). It has been reported that macromolecular MRI contrast agents are effective in assessment of tumor vascularity and characterization of individual tumor malignancy with DCE-MRI (3). DCE-MRI with macromolecular contrast agents of high molecular weight provides more accurate assessment of tumor angiogenesis than the agents with low molecular weights (11–14). Good correlation has also been reported between the histological analysis and tumor vascular permeability estimated by DCE-MRI and macromolecular contrast agent, but not for low molecular weight agents (5). Unfortunately, no macromolecular MRI contrast agents are available for clinical applications because of the safety concerns related to their slow excretion and high tissue accumulation of toxic Gd(III) ions.

We recently developed polydisulfide Gd(III) chelates as biodegradable macromolecular MRI contrast agents to facilitate the excretion of Gd(III) chelates after MRI examinations (15–18). These agents initially behave as macromolecular agents and result in contrast enhancement superior to low molecular weight contrast agents in the vasculature and tumor tissues, suggesting that they can be used for the characterization of tumor angiogenesis with DCE-MRI. They can be readily degraded in vivo into oligomeric and low molecular weight Gd(III) chelates, which rapidly excrete from the body via renal filtration, resulting in minimal tissue accumulation, similar to that of low molecular weight contrast agents (16,19,20). The half-life of the plasma distribution phase (α) of Gd-DTPA cystamine copolymers (GDCC) (60 kDa) and Gd-DTPA cystine copolymers (GDCP) (35 kDa) are 1.74 ± 0.57 and 3.15 ± 1.26 min, respectively (19,20), longer than that of Gadodia-

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Grant sponsor: National Institutes of Health (NIH); Grant numbers: R33 CA095873, R01 EB00489.

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Received 29 January 2008; revised 17 June 2008; accepted 28 July 2008.

DOI 10.1002/mrm.21791

Published online in Wiley InterScience (www.interscience.wiley.com).
mide [Gd (DTPA-BMA)] (0.48 ± 0.16 min) and shorter than a nondegradable macromolecular agent, Gd-DTPA hexanediamine copolymers (35 kDa, 5.92 ± 2.02 min). The long-term Gd tissue accumulation of the biodegradable macromolecular agents is comparable to that of Gd(DTPA-BMA) (19,20). These agents have shown a great promise for further clinical development.

The purpose of this study was to evaluate the potential of the polydisulfide Gd(III) complexes in the characterization of tumor angiogenesis with DCE-MRI in an animal tumor model. A two-compartment model was used for calculating the tumor vascular parameters such as $K_{ps}$, $f_{PV}$, and $PS$ from the DCE-MRI data (6,8,21). The effect of the size and biodegradability of the agents on the vascular kinetic parameters was analyzed and compared to those obtained with Gd (DTPA-BMA) and albumin labeled with Gd-DTPA [albumin-(Gd-DTPA)].

MATERIALS AND METHODS
Contrast Agents
GDCC and GDCP were prepared as previously described (16,22). They were fractionated using a preparative Superose 6 column on an AKTAPrime™plus (GE Biosciences, Piscataway, NJ, USA) to prepare the agents with narrow molecular weight distributions. The average molecular weights of the fractions were determined by size exclusion chromatography using linear poly(N-(2-hydroxypropyl)methacrylamide) as a standard with a analytic Superose 6 column on an AKTA fast protein liquid chromatography (FPLC) system (GE Biosciences). Albumin-(Gd-DTPA) (92 kDa) is a nondegradable macromolecular contrast agent and was prepared according to a published method (23). The Gd(III) content in the agents was determined by inductively-coupled plasma optical emission spectroscopy (ICP-OES) (Optima 3100XL; Perkin Elmer).

Tumor Model and Animal Model
MDA-PCa-2b, a slow-growing prostate adenocarcinoma cell line derived from bone metastasis, was obtained from the ATCC. MDA-PCa-2b cell line derived from bone metastasis, was obtained from the ATCC. Athymic male nu/nu American Type Culture Collection (ATCC number: CRL-2292) of MDA-PCa-2b, a slow-growing prostate adenocarcinoma tumor model and animal model was provided by the ATCC. The Gd(III) content in the agents was determined by inductively-coupled plasma optical emission spectroscopy (ICP-OES) (Optima 3100XL; Perkin Elmer).

DCE-MRI
All images were acquired on a Siemens Trio 3T scanner using the system body coil for RF excitation and a human wrist coil for RF reception. A group of three mice were used for each agent. Mice were anesthetized by intraperitoneal injection of a mixture of ketamine (Bedford, OH, USA; 90 mg/kg) and xylazine (St. Joseph, MO, USA; 10 mg/kg). They were then placed prone with the tumors located at the center of a human wrist. A tail vein of the mouse was catheterized using a 30-gauge needle connected to a 2-m-long thin tubing filled with heparinized saline. Contrast agent solution (120 μl) was injected via the tubing and 200 μl saline was used to flush the tubing after the injection of contrast agents. The dose for all contrast agents was 0.1 mmol Gd/kg except that the dose for albumin-(Gd-DTPA) was 0.03 mmol Gd/kg as reported in the literature.

The 3D FLASH images of mice were acquired using a 3D fast low angle shot (FLASH) sequence (TR/TE = 7.75/2.56 ms, α = 25°, coronal slice thickness = 0.5 mm, averages = 4, fat saturation, and scan time = 1:21 min:s) before contrast administration. The heart and tumor were located at 3D FLASH images. Two-dimensional axial images of the heart and tumor were then acquired using a $T_1$-weighted 2D spin echo (SE) sequence (TR/TE = 400/10 ms, α = 90°, axial slice thickness = 2 mm, averages = 2, fat saturation, slices = 8, and scan time = 1:01 min:s). Dynamic MRI scans were performed with a 2D FLASH sequence (TR/TE = 58/4.03 ms, α = 30°, axial slice thickness = 2 mm, acquisitions = 1, acquisition size = 1 × 1 × 2 mm, axial slices = 6, and scan time = 6 s). The first slice was selected to cover the heart and the rest slices were selected to cover the majority of the tumor tissues. After a 30-s delay, the contrast agent was administered by bolus injection via the catheter. The duration of data acquisition was 45 min for the mice injected with albumin-(Gd-DTPA) and 30 min for the mice injected with all other contrast agents.

Image Analysis
The 3D FLASH and 2D SE images were reconstructed and analyzed using OsiriX (http://homepage.mac.com/rosset-antoine/osiriX). A package of programs based on MATLAB (The MathWorks, Inc., Natick, MA, USA) was developed to process dynamic 2D FLASH data in the digital imaging and communications in medicine (DICOM) format. Regions of interest (ROIs) were placed in the whole tumor and in the right ventricle of the heart to obtain signal intensity (SI) of the blood. The average MR SI of ROI before the contrast agent injection was used as the baseline and was subtracted from the SI after contrast agent injection (SI$_{post}$) to calculate the increase in SI ($\Delta$SI). It is assumed that $\Delta$SI is proportional to the change of the contrast agent concentration, which is a reasonable approximation at low contrast agent concentration (21). A two-compartment model (Fig. 1) consisting of the tumor plasma compartment and the extravascular and extracellular space (EES) was used for analyzing DCE-MRI data (21). The tumor $K_{ps}$, $f_{PV}$, and $PS$ were similarly calculated by the methods as described in the literature (21). Since a high temporal resolution of 6 s per acquisition was used in this study, the maximum blood $\Delta$SI was used directly to calculate the $f_{PV}$. The extrapolation method in case of 1 min per acquisition in the literature (21) is no longer needed.

Statistical Analysis
Statistical analysis was performed using a Student’s $t$-test (GraphPad Prism; GraphPad Software, San Diego, CA,
RESULTS

Contrast Agents

The polydisulfide Gd(III) complexes, GDCC and GDCP, of different molecular weights and narrow molecular weight distribution, were prepared by the fractionation of the polymeric agents using size-exclusion chromatography. GDCP is a modified polydisulfide Gd(III) complex of GDCC with a slower degradation rate (15). Table 1 lists the physicochemical parameters of the contrast agents, including the number average molecular weight ($M_n$), weight average molecular weight ($M_w$), polydispersion index (PDI), and $T_1$ relaxivity per complexed Gd(III) ion at 3T. The agents with molecular weights of 20 and 70 kDa, GDCC-20, GDCC-70, GDCP-20, and GDCP-70, were chosen to represent biodegradable macromolecular MRI contrast agents of relatively low and high molecular weights for studying the size effect. The apparent molecular weights of albumin-(Gd-DTPA) were smaller than its true molecular weight because they were measured as the hydrodynamic volume relative to the linear poly[N-(2-hydroxypropyl)methacrylamide] standards in order to better compare to the linear biodegradable macromolecular contrast agents.

Table 1

Mn, Mw, PDI, and Relaxivity ($r_1$ at 3T) of the Contrast Agents

<table>
<thead>
<tr>
<th>Contrast agent</th>
<th>$M_n$ (kDa)</th>
<th>$M_w$ (kDa)</th>
<th>PDI</th>
<th>Relaxivity ($r_1$) (mM$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd (DTPA-BMA)</td>
<td>0.574</td>
<td>0.574</td>
<td>N/A</td>
<td>4.62</td>
</tr>
<tr>
<td>GDCC-20</td>
<td>19</td>
<td>21</td>
<td>1.10</td>
<td>5.45</td>
</tr>
<tr>
<td>GDCC-20</td>
<td>19</td>
<td>21</td>
<td>1.07</td>
<td>6.04</td>
</tr>
<tr>
<td>GDCP-20</td>
<td>68</td>
<td>73</td>
<td>1.10</td>
<td>5.35</td>
</tr>
<tr>
<td>GDCP-70</td>
<td>68</td>
<td>73</td>
<td>1.07</td>
<td>6.44</td>
</tr>
<tr>
<td>Albumin-(Gd-DTPA)</td>
<td>44</td>
<td>45</td>
<td>1.02</td>
<td>7.05</td>
</tr>
</tbody>
</table>

DCE-MRI

Figure 2a depicts representative kinetics of the MR SI of the blood in the heart before and after injection of the contrast agents. Strong and prolonged enhancement was observed in the blood with the agents of high molecular weights and low degradability. Gd (DTPA-BMA) cleared more rapidly from the blood than the macromolecular contrast agents. GDCC-20 and GDCP-20 reached peak SI about 2 min after injection and started to clear quickly as compared to the corresponding high molecular weight agents. The blood enhancement by GDCC-70 after the peak enhancement decayed more rapidly than that with GDCP-70 because of the high biodegradability of GDCC. The nondegradable control agent, albumin-(Gd-DTPA), maintained a high blood enhancement during the period of data acquisition.

Figure 2b shows the representative kinetics of the MR SI in the tumor tissue. The peak time of SI delayed as the molecular weight of the agents increased and their degradability decreased. The peak enhancement by GDCP-70 was reached about 30 min after injection, while the enhancement of tumor by albumin-(Gd-DTPA) was still increasing at 30 min after injection. The infusion of contrast agents into the tumor EES is controlled by their size and concentration in the blood, and the permeability of the tumor microvasculature. The initial signal rise indicated the plasma volume or EES measured by the contrast agents based on their size-dependent infusion into tumor tissue. With the increasing concentration of the contrast agents in the tumor EES, the signal from the injected contrast agents was observed to be prolonged.
the tumor and the decreasing concentration in the blood due to excretion, the SI in tumor would reach a plateau and then decrease. The larger the contrast agent of the same degradability, the later the plateau was reached.

The kinetic parameters of the tumor vascularity (f_{PV}, K_{PS}, and PS) estimated by DCE-MRI using the biodegradable macromolecular contrast agents and control agents are listed in Table 2. The comparison of the vascular parameters estimated by different contrast agents is shown in Fig. 3. Gd (DTPA-BMA) had the highest f_{PV}, K_{PS}, and PS values, while albumin-(Gd-DTPA) had the lowest f_{PV}, K_{PS}, and PS values. The values estimated by GDCC-20, GDCP-20, GDCC-70, and GDCP-70 were between those by Gd (DTPA-BMA) and albumin-(Gd-DTPA). GDCC and GDCP of 70 kDa had lower values of f_{PV}, K_{PS}, and PS than GDCC. The f_{PV} of MDA-PCa-2b prostate adenocarcinoma xenografts estimated using albumin-(Gd-DTPA) was 0.044 ± 0.007, which was in the same range of reported data (0.034 ± 0.02 and 0.064 ± 0.055) of other tumor models (3,6). The reported K_{PS} with albumin-(Gd-DTPA) varied from tumor models (3,6,24), which was also different from the data in this study, possibly because different tumor models had different vascular permeability.

**DISCUSSION**

This study is the first attempt of using biodegradable macromolecular MRI contrast agents for characterizing tumor angiogenesis with DCE-MRI. Angiogenic tumor vasculature is hyperpermeable to macromolecules. The vascular parameters K_{PS} and PS measured by macromolecular MRI contrast agents with DCE-MRI can represent the tumor vascular permeability and can also be used for quantitative assessment of tumor angiogenesis (8,24). Polydisulfide Gd(III) complexes are promising biodegradable macromolecular MRI contrast agents for further clinical development. The results in this study have shown that the tumor vascular kinetic parameters (f_{PV}, K_{PS}, and PS) measured by DCE-MRI depend on the size and degradability of the agents.

The fractional plasma volume (f_{PV}) determined by GDCC-20 and GDCP-20 was relatively high, which may be attributed to the relatively rapid diffusion of the agents into tumor extracellular space, similar to the low molecular weight agent Gd (DTPA-BMA). It is hypothesized that the high values of f_{PV} obtained with low molecular weight contrast agents are due to their relatively rapid extravasation into the tumor EES (8,24). The rapid uptake of the low molecular weight contrast agents was evidenced by the tumor contrast uptake kinetics (Fig. 2b). The values from GDCC-20 and GDCP-20 may overestimate the tumor fractional plasma volume due to diffusion. This may also be the reason why GDCC-20 and GDCP-20 had similar tumor uptake kinetics, while GDCC-70 and GDCP-70 had different uptake kinetics due to their difference in degradability. The f_{PV} measured by the GDCC-70 and GDCP-70 was relatively low because of limited diffusion due to their large size. The f_{PV} from GDCC-70 and GDCP-70 was slightly higher than that estimated by albumin-(Gd-DTPA) because the low molecular weight degradation products of GDCC-70 and GDCP-70 could diffuse into tumor extracellular space, even though GDCC-70 and GDCP-70 initially had a larger hydrodynamic volume.

The K_{PS} and PS measured by the biodegradable macromolecular contrast agents are significantly smaller than

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**Table 2**

Estimated Values of f_{PV}, K_{PS}, and PS Estimated by DCE-MRI Using Gd (DTPA-BMA), GDCC-20, GDCP-20, GDCC-70, GDCP-70, and Albumin-(Gd-DTPA)*

<table>
<thead>
<tr>
<th></th>
<th>f_{PV}</th>
<th>K_{PS} (ml/min/100 cc)</th>
<th>PS (ml/min/100 cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd DTPA-BMA</td>
<td>0.161 ± 0.018</td>
<td>29.08 ± 10.95</td>
<td>4.809 ± 2.385</td>
</tr>
<tr>
<td>GDCC-20</td>
<td>0.117 ± 0.010</td>
<td>9.98 ± 1.75</td>
<td>1.170 ± 0.209</td>
</tr>
<tr>
<td>GDCP-20</td>
<td>0.112 ± 0.029*</td>
<td>11.08 ± 5.63*</td>
<td>1.343 ± 1.001*</td>
</tr>
<tr>
<td>GDCC-70</td>
<td>0.087 ± 0.005</td>
<td>6.75 ± 0.16</td>
<td>0.586 ± 0.047</td>
</tr>
<tr>
<td>GDCP-70</td>
<td>0.075 ± 0.021</td>
<td>2.09 ± 0.50b</td>
<td>0.161 ± 0.070</td>
</tr>
<tr>
<td>Albumin-(Gd-DTPA)</td>
<td>0.044 ± 0.007</td>
<td>1.43 ± 0.64</td>
<td>0.064 ± 0.029</td>
</tr>
</tbody>
</table>

*The difference is significant when the parameters estimated by the biodegradable macromolecular contrast agents are compared to those by albumin-(Gd-DTPA) and Gd DTPA-BMA, unless indicated otherwise.

*aStatistically not significantly different when compared to Gd (DTPA-BMA).

*bStatistically not significantly different when compared to albumin-(Gd-DTPA).
those determined by Gd (DTPA-BMA), possibly due to their limited diffusion (8,24). The difference in biodegradability between GDCC and GDCP did not affect the $K^{PS}$ and PS measured by GDCC-20 and GDCP-20, possibly because of their relatively high diffusion rate from the blood to tumor extracellular space. For the agents with higher molecular weights, GDCC-70 with high degradability gave higher values for $K^{PS}$ and PS than GDCP-70. Based on the tumor kinetic uptake in Fig. 2b, it is plausible to assume that the rapid degradation of GDCC in the blood plasma resulted in higher uptake rate for GDCC-70 than GDCP-70 due to diffusion of the low molecular weight degradation products into tumor extracellular space. Nevertheless, the parameters measured by the degradable macromolecular MRI contrast agents with high molecular weights were closer to those estimated by albumin-(Gd-DTPA).

The difference of the tumor vascular parameters of the different contract agents can also be expressed as the ratios of the parameters to those measured by albumin-(Gd-DTPA) (Table 3). The ratios might be an indicator of the deviation of these vascular parameters caused by the diffusion of the contrast agents. Gd (DTPA-BMA) had the highest deviation, possibly because of its highest diffusion rate from blood to tumor among the tested agents. The deviation of the biodegradable macromolecular contrast agents with high molecular weight was much smaller than other tested agents. Although it is difficult to know the exact kinetic vascular parameters of tumor tissue, it is reasonable to deduce that GDCP-70 and GDCC-70 can be effective to evaluate tumor angiogenesis with DCE-MRI based on the tumor vascular kinetic parameters.

Noninvasive and accurate evaluation of tumor angiogenesis is critical for tumor characterization and assessment of tumor response to anticancer treatment, particularly anti-angiogenesis therapies. Although it has been demonstrated that DCE-MRI with macromolecular MRI contrast agents is more accurate to evaluate tumor angiogenesis than that with small molecular contrast agents, clinical development of macromolecular contrast agents are limited by their slow excretion and prolonged tissue accumulation. This study has shown that polydisulfide-based biodegradable macromolecular MRI contrast agents are effective for evaluation of tumor angiogenesis with DCE-MRI. One concern for the biodegradable macromolecular contrast agents is the change of the relaxivities of Gd(III) chelates after the degradation of the macromolecules. As shown in this study and our previous studies, the relaxivities of macromolecular contrast agents generally decrease as their molecular weights decrease, but the change of the relaxivities of GDCC and GDCP is not dramatic among the agents of different molecular weights (16). Since DCE-MRI is not a perfectly quantitative method, a slight change in relaxivities due to the degradation of the macromolecules may not be a great concern for assessing tumor angiogenesis. Another concern is the change of pharmacokinetics of lower molecular weight degradation products, which may complicate the kinetic analysis of the DCE-MRI data. As we have shown in this study, although the degradability and size of the biodegradable macromolecular contrast agent affect the vascular kinetic parameters, the parameters estimated by the biodegradable macromolecular contrast agents, particularly those with relatively large sizes, are comparable to those estimated by albumin-(Gd-DTPA). The two-compartment model used here is commonly used for DCE-MRI with macromolecular contrast agents, which is focused on the initial permeation of the contrast agents in the tumor tissue. However, the method used for analyzing the DCE-MRI data is empirical and does not include curve-fitting of the kinetic data. In order to more accurately investigate the effectiveness of the biodegradable macromolecular MRI contrast agents on assessing tumor angiogenesis with DCE-MRI, further study is ongoing to analyze the DCE-MRI data with the biodegradable macromolecular MRI contrast agents using other reported kinetic models.

**CONCLUSIONS**

Polydisulfide-based biodegradable macromolecular contrast agents are effective for evaluation of tumor angiogenesis with DCE-MRI in an animal tumor model. The size of biodegradable macromolecular contrast agents has a significant effect on the vascular kinetic parameters. The degradability only has a significant impact on the agents of the high molecular weights. The biodegradable macromolecular agents with high molecular weights may provide more accurate assessment on tumor vascularity and angiogenesis with DCE-MRI than the low molecular weight agents.

**ACKNOWLEDGEMENTS**

We thank Dr. Yong-En Sun for the animal handling, Melody Johnson for the operation of MRI scanner.

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