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Antiangiogenic Therapy for Primary Liver Cancer: Correlation of Changes in Dynamic Contrast-Enhanced Magnetic Resonance Imaging with Tissue Hypoxia Markers and Clinical Response

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Abstract

Background—This study utilized the imaging data of primary liver cancer (PLC) treated with floxuridine (FUDR) and bevacizumab to test the hypothesis that dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) parameters correlate with tissue hypoxia markers and treatment outcome.

Methods—Seventeen patients with PLC were treated with hepatic artery infusional (HAI) FUDR for 14 days followed by systemic bevacizumab therapy. DCE-MRI images were obtained at baseline and after HAI FUDR and bevacizumab therapy. The parameters (K_{trans} , AUC) pertaining to perfusion and vascular permeability of the tumor and adjacent liver parenchyma were measured with DCE-MRI. Tissue obtained at baseline was stained for hypoxia markers (anti-hypoxia inducible factor-1 α , anti-carbonic anhydrase IX, and vascular endothelial growth factor). Changes in DCE-MRI parameters were correlated with tissue hypoxia and time to progression (TTP).

Results—The median TTP was 8.8 months. Significant decreases in AUC90 ($P = 0.004$), AUC180 ($P = 0.004$), and K_{trans} ($P = 0.05$) were noted in tumors after bevacizumab but not in nontumor areas. TTP correlated inversely with changes in AUC90 and AUC180 after bevacizumab ($P = 0.002$ and $P = 0.0001$). Reductions in tumor perfusion (AUC90 and AUC180) were greater in tumors expressing anti-hypoxia inducible factor-1 α ($P = 0.02$ and 0.03), vascular endothelial growth factor ($P = 0.01$ and $P = 0.01$), and anti-carbonic anhydrase IX ($P = 0.009$ and $P = 0.009$).

Conclusions—In patients with PLC, bevacizumab induces a reduction in tumor perfusion measured by DCE-MRI. These changes correlate with TTP and tissue markers of tumor hypoxia.

Tumor response to cytotoxic chemotherapy is typically measured on cross-sectional imaging using either the Response Evaluation Criteria in Solid Tumors (RECIST) or World Health Organization (WHO) criteria. However, these standard response criteria correlate poorly with outcome in patients treated with antiangiogenic and other targeted therapies, as demonstrated in recent clinical trial of patients with primary liver cancer (PLC).¹ Conventional imaging studies provide information regarding tumor anatomy, morphology, and to a much lesser extent, physiology; however, the concept of using imaging to characterize tumors at the molecular biologic level has only recently emerged.²⁻⁴

Primary liver cancer (PLC) consists almost entirely of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) and is among the most common solid tumors, ranking behind only lung cancer and gastric cancer in worldwide cancer-related mortality.⁵ Patients with unresectable disease have few treatment options, with a median survival of 12 months or less.^{6,7} Despite a recent study demonstrating clinical benefit of the multikinase inhibitor sorafenib in treating advanced HCC, reliable biomarkers that predict treatment response and influence clinical decision making are not defined and remain a major limitation of this therapy, and indeed of most biological or cytostatic agents.¹

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) noninvasively assesses the tumor microvasculature, allowing quantitative and semi-quantitative measurements of kinetic parameters related to tumor perfusion and vascular permeability.⁸ Previous studies have suggested that changes in DCE-MRI kinetic parameters occur after antiangiogenic therapy but few have correlated these changes with other nonimaging biomarkers or with clinical outcome.^{9,10} Thus far, DCE-MRI after antiangiogenic therapy has proven useful as a pharmacodynamic biomarker, but data showing utility as a predictor of outcome are lacking.

Tumor expression of vascular endothelial growth factor (VEGF) and other pro-angiogenic factors result in abnormal tumor vasculature and a microenvironment characterized by increased vascular density and vascular permeability, increased interstitial pressure and hypoxia.¹¹⁻¹⁵ Antiangiogenic drugs have been postulated to reverse these changes by “normalizing” the tumor microvasculature, thereby improving blood flow, reducing hypoxia and possibly enhancing the delivery and impact of therapeutic agents.¹⁶ Standard imaging studies and response measures, which are based on changes in tumor size, are ill-equipped to assess these effects and underestimate the impact of these agents.¹⁷⁻¹⁹

Using a model of PLC with a treatment paradigm of hepatic arterial infusion (HAI) of floxuridine (FUDR) and dexamethasone (dex) in combination with systemic bevacizumab, this study investigates tumor kinetic parameters assessed by DCE-MRI and hypoxia tissue markers, as potential biomarkers of antiangiogenesis therapy and predictors of treatment outcome. The use of DCE-MRI and tissue markers seeks to establish a correlation between diagnostic imaging and molecular diagnostics.

METHODS

Patients

This phase II trial was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center. All patients had histologically proven ICC or HCC determined to be unresectable by attending hepatobiliary surgeons. Exclusionary factors have been described previously.²⁰ Patients with presumed ICC also underwent esophagogastroduodenoscopy, colonoscopy, and mammography (female subjects).

The hepatic artery infusion pump (HAIP) was placed intraoperatively as previously described.²¹ Tumor and liver biopsies were performed as part of the procedure. Tumor and liver biopsies were not performed during the course of treatment. Pretreatment MRI scans were performed with conventional and dynamic sequences and were repeated every 14 days for the first 28 days and every other month thereafter while patients were enrolled on the protocol. The RECIST criteria were used to categorize responses and to determine time to progression (TTP) on the basis of tumor size measurements using conventional MRI sequences and confirmed by the study radiologist (LS).²²

Chemotherapy Administration

All study patients enrolled in the study received HAI FUDR ($0.16 \text{ mg/kg} \times 20/\text{pump flow rate}$) and dex (1 mg/m^2 daily) no sooner than 14 days after pump placement. After 14 days of FUDR/dex, the HAIP was emptied and filled with heparinized saline, and systemic bevacizumab treatment (5 mg/kg as a single dose) was administered. After an additional 14 days, FUDR/dex was again administered, and bevacizumab was provided every other week. The cycle was repeated every 28 days until disease progression, resection, or toxicity mandated removal from the study. Toxicity was graded according to the National Cancer Institute, Common Toxicity Criteria; the FUDR dose was adjusted as previously described.²³ The end points of treatment were disease progression, excessive toxicity, and achievement of resectability.

DCE-MRI

DCE-MRI scans were obtained before the onset of FUDR therapy (Tx0), after 14 days of HAI FUDR/dex therapy (Tx1), and 14 days after a single dose of systemic bevacizumab (Tx2). DCE-MRI parameters in the tumor and the adjacent nontumor hepatic parenchyma were measured and analyzed at each time point; the interval Tx0–Tx1 refers to the measurements made at the completion of initial treatment with FUDR/dex only and Tx1–Tx2 refers to measurements made 14 days after a single dose of bevacizumab. DCE-MRI data were not included for one patient whose first dose of bevacizumab was delayed as a result of an infection.

DCE-MRI scans were acquired using a 1.5-T MRI scanner (GE Medical Systems, Waukesha, WI) and an 8-channel phased array coil using previously described MRI parameters and contrast infusions.^{24,25} Anatomical images, fat-suppressed T1- and T2-weighted images, three-dimensional T1-weighted pre- and postcontrast images, and fast gradient echo-based perfusion images were acquired during each scan. Paramagnetic contrast Gd-DTPA (gadolinium diethylenetriaminepentaacetic acid; Magnevist, Berlex Inc., Wayne, NJ) was administered at a constant dose of 0.1 mmol/kg body weight. A bolus of gadolinium was injected with a delay of 6 s using automatic power injector (Medrad, Warrendale, PA) and serial images with a temporal resolution of 1 s were acquired over 4–5 min under shallow breathing conditions. One coronal oblique plane was prescribed through the approximate center of the tumor and the aorta to maximize the cross-sectional area and the temporal resolution. The nominal DCE-MRI parameters: echo time (TE) = 2 ms, repetition time (TR) = 8 ms, flip angle = 30° , slice thickness = 7 mm, matrix size (M) = 256×128 , field of view (FOV) = 320 mm, bandwidth (BW) = 23.8 kHz, number of excitations (NEX) = 1.0, and number of phases (n) = 225. Images were obtained pretreatment (Tx0), after 14 days of HAI FUDR/dex therapy (Tx1) and 14 days after a single dose of systemic bevacizumab therapy (Tx2). Dynamic images were analyzed using a two-compartmental general kinetic model with a vascular space and an extravascular and extracellular space for pharmacokinetic characterization of tumors and adjacent non-tumor-bearing liver parenchyma.^{26,27} An average biexponential vascular input function was used for analysis (Cine Research Software, GE Medical Systems, Waukesha, WI).^{24,25,28,29} The variables

measured included: volume transfer constant (K_{trans} , 1/min) from vascular to extracellular space, the rate constant (k_{ep} , 1/min) from extracellular to vascular space, fractional extracellular space volume (v_e), fractional plasma volume (v_p), and the integrated area under the concentration curve of gadolinium contrast agent over 90 and 180 s from the start of contrast enhancement (AUC90 and AUC180, mM•s). In each image, region of interest (ROI) covering the tumor and voxels (V_x) within the ROI were analyzed to determine the kinetic parameters of the tumors or the normal surrounding tissue. For the V_x analysis, the top decile values corresponding to the viable tumor within the ROI were used as the variable of interest. The change in DCE-MRI parameters from baseline (Tx0) to the first (Tx1) and second (Tx2) posttreatment scans was determined using ROI (AUC90 and AUC180) or top decile V_x values (K_{trans}).

Immunohistochemistry

Assessment of baseline tumor hypoxia was performed using immunohistochemistry on 4- μ m-thick sections of paraffin-embedded tissue. The primary antibodies were anti-hypoxia inducible factor-1 α (HIF-1 α) at 1:1600 dilution (Novus Biologicals, Littleton, CO), anti-carbonic anhydrase IX (CA IX) at 1:600 dilution (Novus Biologicals), anti-VEGF-A at 1:100 dilution (Santa Cruz Biotechnology, Santa Cruz, CA). For HIF-1 α , a Catalytic Signal Amplification kit (Dako, Carpinteria, CA) was used to intensify the signal. The peroxidase reaction was developed with DAB staining. For negative control, the primary antibodies were replaced by nonimmune serum. All immunostains were evaluated without any knowledge of the clinical findings by a single pathologist (JS). Stains were graded according to the percentage of positive tumor cells. For HIF-1 α and CA IX, positive staining required positive nuclear labeling in >5% of tumor cells, and for VEGF, positive staining refers to cytoplasmic and/or membranous labeling in >10% cells.

Statistics

Progression-free survival was measured from treatment start date to first documented progression, death or last follow-up and estimated by the Kaplan-Meier method. Changes in DCE-MRI kinetic parameters and serum and tissue angiogenesis and hypoxia markers were compared using the Wilcoxon signed rank test. Correlation of time to progression with DCE-MRI kinetic parameters and serum markers of angiogenesis was assessed using the Somers' D rank correlation. To handle censoring properly, concordance (c) from a Cox model was estimated and then converted it to Somers' D using $D = 2 \times (c - 0.5)$.³⁰ Correlation between DCE-MRI parameters and changes in circulating angiogenic markers assessed using Spearman's rho. Statistical analysis was performed using the SPSS statistical software package (Version 17.0 for Macintosh, SPSS Inc, Chicago, IL) and R (version 2.10, www.r-project.org).

RESULTS

Patient Characteristics and Follow-up

Between August 2007 to January 2009, 17 patients with histologically proven, unresectable PLC (HCC ($n = 3$) or ICC ($n = 14$)) were included in the protocol. The median age was 62 years (range = 46 to 84 years) and 10 patients (57%) were women. At a median follow-up time of 19 months (range = 1.8 to 27.2 months), the overall TTP was 8.8 months (range = 1.8 to 21.2).

Change in DCE-MRI Parameters with Chemotherapy

After the initial HAI FUDR/dex therapy (Tx0-Tx1), tumors showed no statistically significant changes or trends in any of the measured DCE-MRI parameters, compared to

baseline (Fig. 1). By contrast, 14 days after a single dose of systemic bevacizumab therapy (Tx1–Tx2), tumors showed significant decreases in AUC90 (median change, -29.9% , range $+16.7\%$ to -74.6% , $P = 0.004$), AUC180 (median change, -25.8% , range $+14.1\%$ to -75.2% , $P = 0.002$) and K_{trans} (median change, -24% , range $+45.44\%$ to -89.1% , $P = 0.05$). Adjacent nontumor areas of the liver, on the other hand, showed no statistically significant changes in any of the kinetic parameters, neither after initial HAI FUDR/dex treatment nor after systemic bevacizumab (Fig. 1). The statistically significant changes in measured DCE-MRI parameters were independent of diagnosis, and both HCC and ICC subtypes demonstrated similar results.

Changes in DCE-MRI Parameters and Clinical Outcome

A significant inverse correlation was observed between TTP and the changes in AUC90 and AUC180 14 days after systemic bevacizumab treatment ($D = -0.51$, $P = 0.002$ and $D = -0.56$, $P = 0.0001$, respectively) (Fig. 2). There were no statistically significant correlations between TTP and changes in AUC90, AUC180, and K_{trans} after the initial HAI FUDR/dex therapy or between overall TTP and K_{trans} after bevacizumab therapy. Likewise, progression free survival was inversely related to changes in AUC90 and AUC180 after completion of systemic bevacizumab therapy. For this analysis, patients were dichotomized around the median change in AUC90 (-29.9%) and AUC180 (-25.7%) from Tx1 to Tx2. For both AUC90 and AUC180, a greater decrease in both of these variables was associated with longer TTP compared to reductions of lesser magnitude (or increases) (Fig. 3). For AUC90 values of -29.9% or less, TTP was 9.6 months compared to only 5.4 months if it was greater than this median value ($P = 0.05$). A similar association was observed in patients with a change in AUC180 of -25.7% or less (i.e., a greater decrease) compared to a change greater than -25.7% (11.0 months and 5.4 months, respectively, $P = 0.004$) (Fig. 3). The statistically significant correlations in measured DCE-MRI parameters and TTP were independent of diagnosis.

Tissue Markers of Hypoxia and Angiogenesis

To determine the relative impact of antiangiogenic therapy in hypoxic tumors, where the tumor vasculature is least likely to resemble normal vascular beds, DCE-MRI kinetic parameters over time were stratified by baseline tumor expression of the hypoxia markers, HIF-1 α , CA IX and VEGF, as determined by immunohistochemistry (Table 1). In patients with HIF-1 α -positive tumors ($n = 6$), AUC90 (change in median value from 15.8 mM•s to 10.7 mM•s) and AUC180 (change in median value from 35.7 to 29.3) both decreased significantly after bevacizumab therapy (Tx1–Tx2) ($P = 0.02$ and $P = 0.03$, respectively). Similarly significant decreases in AUC90 (change in median value from 20.5 to 10.5) and AUC180 (change in median value from 49.4 to 29.3) were observed in VEGF-positive ($P = 0.01$ and $P = 0.01$, respectively) tumors. CA IX-positive tumors ($n = 10$) exhibited similarly significant decreases in both AUC90 and AUC180 ($P = 0.009$ and $P = 0.009$, respectively) (data not shown). By contrast, tumors that failed to express HIF-1 α ($n = 9$), VEGF ($n = 5$) or CA IX ($n = 4$) showed no significant changes in AUC90 or AUC180 after bevacizumab. No changes in K_{trans} after bevacizumab therapy were observed after initial HAI FUDR/dex therapy or after a single dose of bevacizumab, regardless of whether the tumors were positive or negative for HIF-1 α , VEGF, or CA IX. These findings were independent of histopathological diagnosis of ICC or HCC.

DISCUSSION

This study used an in vivo primary liver tumor model to demonstrate a link between diagnostic imaging and molecular diagnostics. Primary liver tumors exhibit a high degree of abnormal tumor vasculature, making them potentially good targets for antiangiogenic

therapy.³¹ Although an emerging field, radiogenomics best defines the study's hypothesis that early posttreatment changes in tumor perfusion kinetics, as measured with DCE-MRI, can be successfully linked to tumor markers of hypoxia and can be used to assess treatment outcome.

Recent studies suggest that antiangiogenic therapy produces a time-sensitive normalization of tumor vasculature by decreasing vascular permeability, reducing interstitial pressure and reversing tumor hypoxia, thereby improving delivery of cytotoxic chemotherapy.^{12–17} In this study, we demonstrated that systemic bevacizumab therapy statistically significantly alters the baseline perfusion kinetics of tumor tissue, as manifested by measureable reductions in the DCE-MRI parameters AUC90, AUC180, and K_{trans} , while at the same time having little impact on the hepatic parenchyma. These results are in line with other published reports showing that decreases in AUC90, AUC180, and K_{trans} are associated with reduced tumor vascular density and vascular permeability and are pharmacodynamic biomarkers of antiangiogenesis therapy.^{32,33}

Beyond their pharmacodynamic relevance, these measureable changes in tumor perfusion kinetics had important clinical implications, as evidenced by their correlation with treatment outcome. Specifically, the magnitude of the reduction in AUC90 and AUC180 correlated statistically significantly with longer TTP. Although, K_{trans} also decreased significantly after bevacizumab therapy, correlation with TTP was not observed. This may be explained by the fact that AUC90 and AUC180 are semiquantitative parameters that are easily calculated and reproducible unlike K_{trans} , a quantitative measure determined by kinetic modeling making reproducibility a limitation.³⁴

It is particularly noteworthy that the bevacizumab-induced changes in perfusion kinetics were more pronounced in tumors that were relatively more hypoxic. Tumor vasculature is structurally abnormal, lacking normal endothelial coverage with pericytes, leading to increased vascular permeability, impaired flow and hypoxia.^{35,36} After a single dose of bevacizumab, tumors expressing the hypoxia markers HIF-1 α , VEGF, and CA IX had statistically significantly greater reductions in AUC90, AUC180, and K_{trans} compared to tumors that did not. From a mechanistic standpoint, it would appear to make sense that bevacizumab would have a greater impact on tumors with a relatively greater degree of hypoxia and vascular beds that are likely to be more structurally abnormal. This is further supported by the observation that the kinetic parameters in the nontumor liver parenchyma did not change significantly, suggesting that the functionally normal vasculature in these areas was not susceptible to modification with bevacizumab.

The data thus support the hypothesis that bevacizumab, through a mechanism of relative normalization of tumor vasculature, enhances delivery of cytotoxic drug. The potential clinical implication of this change is more effective tumor therapy leading to an improvement in outcome. Importantly, improvement in TTP was observed only in patients with decreases in AUC90 and AUC180 in response to a single dose of bevacizumab. These initial changes were measureable using DCE-MRI techniques and were predictive of outcome. The results therefore show the potential utility of DCE-MRI for monitoring antiangiogenic therapy and as a biomarker of therapeutic response.

Not surprisingly, the underlying diagnosis (ICC or HCC) was not a confounding factor in any of the analyses performed. Although HCC is considered more vascular than ICC, the vascular beds in both types are abnormal and both rely on tumor-directed neovascularization for proliferation.³⁷ Because the analyses examined changes from baseline within individual tumors, it is reasonable to include both groups in a single analysis; the fact that the results did not change when HCC and ICC were analyzed separately justifies this decision.

In summary, the results of this phase II trial of patients with advanced PLC showed that bevacizumab induces changes in tumor perfusion kinetics that are measureable using DCE-MRI. These changes in DCE-MRI parameters appear to reflect a normalization of the deranged tumor vasculature, resulting in improvement in hypoxia, and possibly more effective delivery of cytotoxic drug, ultimately leading to longer TTP. The bevacizumab-related changes in tumor perfusion kinetics closely correlated with expression of hypoxia markers within the tumor. The results demonstrate a link between diagnostic imaging and molecular diagnostics and provide a platform to aid clinical decision-making.

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FIG. 1. Effect of HAI FUDR/dex hepatic artery infusion chemotherapy and bevacizumab on tumor and nontumor vascular perfusion. Waterfall plots show the percent change in AUC90, AUC180, and K^{trans} that occurred for all 17 patients after HAI FUDR/dex chemotherapy (Tx0 to Tx1) and 14 days after a single dose of systemic bevacizumab therapy (Tx1 to Tx2)

FIG. 2.

A greater decrease in AUC90 and AUC180 14 days after a single dose of systemic bevacizumab (Tx1 to Tx2) is correlated with overall TTP

FIG. 3. Kaplan-Meier estimated progression-free survival curves of AUC90 and AUC180 stratified by median change in AUC90 and AUC180 14 days after a single dose of systemic bevacizumab. For both AUC90 and AUC180, a greater decrease in both of these variables was associated with longer TTP compared to reductions of lesser magnitude (or increases)

TABLE 1
Decreased vascular permeability after bevacizumab therapy is a function of tumor hypoxia^a

Characteristic	Before therapy (Tx0)			HAI FUDR/dex (Tx1)			Bevacizumab (Tx2)		
	Median (mM s)	Range (mM s)	P*	Median (mM s)	Range (mM s)	P*	Median (mM s)	Range (mM s)	P*
Tissue marker AUC90									
HIF-1 α positive (n = 6)	13.7	9.7–35.5	NA	15.8	9.3–46.2	0.05	10.7	6.9–37.1	0.02
HIF-1 α negative (n = 9)	17.1	7.3–23.9	NA	15.2	11.1–25.2	0.95	13.9	7.8–19.3	0.18
VEGF- α positive (n = 10)	16.5	9.7–35.5	NA	20.5	9.7–35.5	0.24	10.5	7.8–37.1	0.01
VEGF- α negative (n = 5)	17.1	7.3–23.9	NA	15.2	9.3–25.2	0.69	14.2	6.9–19.3	0.43
Tissue marker AUC180									
HIF-1 α positive (n = 6)	29.9	19.4–71.4	NA	35.7	25.9–93.0	0.08	29.3	19.2–75.2	0.02
HIF-1 α negative (n = 9)	38.1	18.7–53.9	NA	36.1	24.3–52.5	0.86	29.3	18.1–38.9	0.08
VEGF- α positive (n = 10)	37.1	19.4–71.4	NA	45.4	24.3–93.0	0.14	29.3	18.1–75.2	0.01
VEGF- α negative (n = 5)	25.9	18.7–53.9	NA	37.3	25.9–52.5	0.50	36.0	19.2–42.4	0.43

HAI/hepatic artery infusion, FUDR floxuridine, dex dexamethasone, HIF-1 α anti-hypoxia inducible factor-1 α , VEGF vascular endothelial growth factor, Tx0 onset of FUDR therapy, Tx1 after 14 days of HAI FUDR/dex therapy, Tx2 and 14 days after a single dose of systemic bevacizumab

^a A significant decrease in AUC90 (mM s) and AUC180 (mM s) upon bevacizumab therapy, the difference during Tx1 and Tx2, is only observed in tumors that stain positive for HIF-1 α and VEGF by immunohistochemistry

* P value calculated by Wilcoxon signed rank test to previous value (i.e., Tx2 value compared to Tx1 value)