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Short Communication

Perfusion imaging of colorectal liver metastases treated with bevacizumab and stereotactic body radiotherapy

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ABSTRACT

Stereotactic body radiotherapy (SBRT) and bevacizumab are used in the treatment of colorectal liver metastases. This study prospectively evaluated changes in perfusion of liver metastases in seven patients treated with both bevacizumab and SBRT. Functional imaging using dynamic contrast-enhanced CT perfusion and contrast-enhanced ultrasound were performed at baseline, after bevacizumab, and after SBRT. After bevacizumab, a significant decrease was found in permeability (-28%, $p < .05$) and blood volume (-47%, $p < .05$), while SBRT led to a significant reduction in permeability (-22%, $p < .05$) and blood flow (-37%, $p < .05$). This study demonstrates that changes in perfusion can be detected after bevacizumab and SBRT.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer death worldwide [1]. Approximately 25–50% of patients with colorectal cancer will eventually have tumor recurrence in their liver [2]. In patients with resectable solitary liver metastases, five year survival rates of 30–40% have been reported [3,4]. Stereotactic body radiotherapy (SBRT) is an alternative to surgical resection of liver metastases [5,6] that has an 84% rate of local control at 18 months [7]. Bevacizumab, a vascular endothelial growth receptor (VEGF) inhibitor, when added to conventional chemotherapy has significantly improved overall survival in patients with metastatic colorectal cancer [8–10]. Bevacizumab improves pathological complete response in patients with rectal cancer when combined with neoadjuvant chemoradiation [11].

Conventional morphologic imaging with magnetic resonance imaging (MRI), ultrasound (US) or computed tomography (CT) is the current standard for diagnosing and monitoring colorectal cancer and liver metastases. In the era of high dose radiation and molecular-based targeted therapies, there is increasing interest in the use of functional imaging as a method to both evaluate and predict response to treatment [12–14]. Perfusion imaging with dynamic contrast enhanced computed tomography (DCE-CT) has been used to assess tumor vascularity and has shown promise in identifying tumors that respond poorly to neoadjuvant chemoradiation [15]. In human colon cancer xenografts in mice, DCE-CT has been used to track changes in perfusion over time after treatment with both bevacizumab and radiation [16], but quantitative perfusion changes in colorectal liver metastases in humans has not been reported to date.

Functional imaging using novel ultrasound (US) techniques have more recently been investigated for characterizing malignant lesions. Contrast-enhanced US (CEUS) is a method that uses a microbubble contrast agent to image flow in the capillary microcirculation [17] that has been used to characterize vascular properties of liver lesions [18]. Preliminary data suggests that microbubble-based ultrasound imaging can improve the detection of small colorectal liver metastases [19].

This prospective pilot study was conducted to evaluate the utility of CT and US perfusion imaging in patients with colorectal liver metastases treated with bevacizumab and SBRT.

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Table 1
Perfusion parameters at baseline, after bevacizumab (and prior to SBRT), and after SBRT for all seven patients. Also shown is the initial tumor volume as well as local or distant recurrence by patient.

Patient #	Tumor size (cc)	Permeability (mL/100 g/mL)				Blood volume (mL/100 g)				Blood flow (mL/100 g/min)				Tumor recurrence
		Baseline T1	Pre SBRT T2	Post SBRT T3	Change (%) (T1-T2/T2-T3)	Baseline T1	Pre SBRT T2	Post SBRT T3	Change (%) (T1-T2/T2-T3)	Baseline T1	Pre SBRT T2	Post SBRT T3	Change (%) (T1-T2/T2-T3)	
1	29	48	43	29	$-10/-33$	21	17	6	$-21/-61$	267	125	79	$-53/-37$	None
2	154	70	69	64	$-1/-7$	44	24	58	$-45/142$	197	141	117	$-29/-17$	Local, distant
3	28	19	14	11	$-27/-22$	23	12	12	$-48/0$	120	99	36	$-18/-64$	Local
4	52	25	18	13	$-28/-28$	100	20	8	$-80/-80$	114	113	42	$-1/-63$	None
5	26	xx	42	12	xx/-70	xx	29	11	xx/-63	xx	394	196	xx/-50	Distant
6	68	32	25	25	$-22/1$	14	10	14	$-27/43$	76	116	90	$52/-23$	Local
7	14	25	6	17	$-75/166$	11	5	5	$-55/0$	117	74	64	$-37/-13$	Distant
				Median change	$-28/-22$				$-47/0$				$-24/-37$	
				W-value	$0^*/1.5^*$				$0^*/6$				$3/0^*$	

[*] Indicates statistical significance at p < .05.

2. Material and methods

Our Institutional Research Ethics Committee approved this study, and informed consent was obtained from each patient. Patients were included if they had one to three liver metastases and histological confirmation of colorectal cancer. Ten patients were enrolled and a total of seven patients (each with a solitary metastasis) were included in the final CT perfusion parametric analysis. CEUS images were acquired in four patients. Baseline patient characteristics are shown in Supplementary Table S1.

Bevacizumab was administered at a dose of 5 mg/kg IV for two doses two weeks apart, starting two weeks before SBRT; the second dose was administered no more than 48 h before starting SBRT. The radiation simulation process has been described previously and contouring was performed on a 4D-CT simulation scan (in the portal-venous phase) to account for respiratory motion [20]. The prescription dose was determined by the volume of liver receiving less than 15 Gy and dose constraints to surrounding organs at risk (median dose 54 Gy, range 36–60 Gy).

All imaging studies were performed at three time points. A baseline scan was performed prior to any treatment. A second scan, after bevacizumab but before SBRT, was obtained within 48 h after the second dose of bevacizumab. The last scan was performed within seven days after completion of SBRT. The DCE-CT was acquired using a 64-slice clinical CT (VCT Lightspeed, GE Medical Systems, Milwaukee, WI) with a field-of-view of 40 cm. Patients then received bolus intravenous CT contrast (Ultravist 370) at a dose of 100 mL at a rate of 4 mL/s, and high temporal resolution scans and time attenuation curves were collected. Data were analyzed using commercially available software (CT Perfusion 4.0, GE Medical Systems). The metastases being treated were contoured on conventional contrast-enhanced CT images by an experienced abdominal radiologist (LM). The Johnson and Wilson model for distribution of CT contrast medium was used [21]. The perfusion analysis is described in detail elsewhere [15]. The DCE-CT output parameters are the following: blood volume (mL/100 g), blood flow (mL blood/100 g/min) and permeability surface area (mL/100 g/mL).

For CEUS, microvascular volume was measured using an approved microbubble agent, Definity (Bristol-Myers Squibb, Boston MA), using bolus and disruption-replenishment methods with pulse inversion contrast-specific imaging software (iU22, Philips Medical Systems or Aplio 80, Toshiba Medical Systems). Following bubble disruption, replenishment into the imaging plane of interest in the tumor was used to calculate the integrated contrast signal, normalized with respect to the signal in the adjacent normal liver. A quantitative perfusion index was calculated as the ratio of the integrated signal to the mean transit time.

The perfusion parameters were averaged over the tumor of interest for each patient and compared longitudinally over the three time points (comparing baseline to post-bevacizumab, and pre-SBRT to post-SBRT) using the Wilcoxon Signed-Rank test as the limited sample size does not allow for the assumption of normality. The output W from the Wilcoxon test is considered to demonstrate a statistically significant difference at a level of 0.05 if W is less than or equal to zero (for n = 6) or W less than or equal to two (for n = 7) [22]. All statistical tests were performed using Matlab (The Mathworks, Natick, MA).

3. Results

The median age of the enrolled patients was 70 years. The mean target volume was 43 cc (range from 14 to 154 cc). During treatment, two patients experienced grade 2 toxicities (fatigue and nausea), while one patient developed grade 3 hypertension. Otherwise there were no other acute or late grade 3–5 toxicities observe during a median follow-up of 412 days. Of the seven evaluable patients with CT perfusion data, three had local failure at the time of last follow up. One of three patients with local failure had simultaneous distant failure; two of the four patients with no local failure developed distant failure. Median overall survival was not reached during follow-up.

The mean permeability decreased in all six patients from baseline to post-bevacizumab (median change -28%, W = 0, p < .05), as did the blood volume (median change -47%, W = 0, p < .05). Blood flow decreased in five of six patients in this cohort (median change -24%, W = 3, p > .05); the one patient that had an increase in blood flow after bevacizumab was one of the three patients that had local failure. CT perfusion data at baseline and after bevacizumab for all patients is shown in Table 1. The changes in perfusion parameters before and after SBRT are shown in Table 2. Radiation caused a decrease in permeability

Table 2
Contrast-enhanced ultrasound (CEUS) perfusion index for individual patients at baseline, after bevacizumab, and after SBRT.

Patient	Baseline (T1)	Post bevacizumab (T2)	Post SBRT (T3)	% change (T1-T2)	% change (T2-T3)
1	0.160	0.090	0.070	-46%	-14%
2	0.070	0.004	0.001	-95%	-79%
3	0.060	0.008	0.005	-86%	-41%
4	0.007	0.001	0.001	-82%	-2%
Median change				-84%	-28%

(median change −22%, W = 1.5, p < .05) and blood flow (median change −37%, W = 0, p < .05). Blood volume was not affected by SBRT (median change 0%, W = 6, p > .05). There were no statistically significant differences in any baseline perfusion parameters or in temporal changes in perfusion parameters after treatment between patients with local control versus those who locally progressed. In the four patients who completed CEUS, the perfusion index decreased from baseline to post-bevacizumab (median change −84%). There was a further small reduction in perfusion after SBRT (median change −28%).

4. Discussion

This study expands on the previous work in a mouse model of colon cancer xenografts [16] by evaluating CT perfusion and CEUS to track changes in colorectal liver metastases treated with bevacizumab and SBRT. Perfusion changes allow post-treatment assessment of differences in tumor perfusion before any changes in anatomical tumor size and may serve as an early predictor of response [23]. DCE-CT of liver metastases from various primary tumors confers prognostic information with higher arterial perfusion correlated with improved survival [24]. Previous studies have shown decreases in blood flow and blood volume in liver metastases in patients treated with another antiangiogenic agent (SU6668) [25] or with cytotoxic chemotherapy [12,26]. Metastatic carcinoid tumors and hepatocellular carcinoma treated with bevacizumab were also shown to have a significant reduction in blood flow and blood volume in two separate studies [27,28]. Preclinical animal studies have also been used to demonstrate decreased perfusion in response to transarterial chemoembolization [29,30].

Similar to the current study, Ren et al. evaluated CT perfusion changes after bevacizumab and high dose single fraction radiation in a human colon cancer xenograft in mice [16], although in their experiment the mice were exposed to *either* bevacizumab or radiation, not both sequentially. Our results are concordant with the results described by Ren in that permeability, blood flow, and blood volume all decrease after treatment with bevacizumab. In their mouse model evaluating perfusion at four time points in the first seven days after a single fraction of high dose of radiation, perfusion parameters showed a complicated time course but the immediate and late (seven day) trends were decreases in permeability, blood flow and blood volume [16]. In the current study, perfusion was only evaluated once within seven days after the completion of a six-fraction course of SBRT and patients were scanned as early as one day and as late as six days after completing radiation (and they were all treated with bevacizumab prior to starting radiation). This makes a direct comparison difficult, though we do note a significant decrease in permeability and blood flow after radiation in the current study that was also seen in the mouse model [16]. We did not detect any trends in blood volume after SBRT which may be due to the temporal evolution of changes in blood volume not detected by imaging at a single time point. Using CT perfusion of primary rectal tumors, Sahani et al. showed that blood flow was reduced after chemoradiation, but blood volume remained the same [15], similar to what was seen in our study. With only seven patients that could be evaluated using CT perfusion, we could not detect any differences in perfusion changes between liver metastases that did or did not fail locally. It has been shown that higher baseline permeability led to improved response rates to combination chemotherapy with bevacizumab in colorectal liver metastases [31]. A larger reduction in blood flow and permeability was also seen in tumors that responded to chemotherapy compared to those that did not respond [12]. These prior studies suggest that with a larger patient population, perfusion parameters may be able to predict clinical outcomes such as time to progression after treatment.

CEUS perfusion imaging was only obtained in four patients in this study. While CEUS did detect decreases in the perfusion index after bevacizumab and to a lesser degree after radiation, the utility of ultrasound-based perfusion parameters is limited unless technical advances are made to improve the image quality for these hypovascular colorectal liver metastases.

A recent review of retrospective studies suggested an increased risk of up to 29% of severe late GI toxicities after combined anti-angiogenics and SBRT [32]. In this small prospective cohort, the combination of bevacizumab and SBRT to colorectal liver metastases was well tolerated without any serious adverse events.

This study has several limitations. The small patient cohort limits the generalizability and strength of any conclusions drawn and should therefore be seen as hypothesis generating to stimulate interest in further study of perfusion imaging of colorectal liver metastases. While the timing of the bevacizumab and SBRT was well defined, the final imaging study was performed over a range of days within a week of completing radiotherapy; this limits the ability to describe the effects of radiation on various perfusion parameters as they exhibit a complicated time course after radiotherapy [16]. Finally, there is a lack of standardization of DCE-CT acquisition and post-processing [23] which needs to be addressed before larger studies can be generalized and widely used.

In conclusion, CT perfusion may be useful in tracking changes in tissue perfusion to colorectal liver metastases treated with systemic or radiation therapy. Further study of DCE-CT in a larger cohort is needed to better understand temporal changes in perfusion, as well as to determine if any differences in these imaging parameters may be useful in predicting response to treatment.

Conflict of interest

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phro.2018.01.001.

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