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Radiofrequency Ablation:Post-ablation Assessment Using CT Perfusion with Pharmacological Modulation in a Rat Subcutaneous Tumor Model

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Abstract

Rationale and Objectives—Inflammatory reaction surrounding the ablated area is a major confounding factor in the early detection of viable tumor after radiofrequency (RF) ablation. A difference in the responsiveness of normal and tumor blood vessels to vasoactive agents may be used to distinguish these regions in post-ablation follow-up. The goal of this study was to examine longitudinal perfusion changes in untreated viable tumor and the peripheral hyperemic rim of RF-ablated tumor in response to a vasoconstrictor (phenylephrine) or vasodilator (hydralazine) in a subcutaneous rat tumor model.

Materials and Methods—Bilateral subcutaneous shoulder tumors were inoculated in 24 BDIX rats and evenly divided into two groups (phenylephrine and hydralazine groups). One tumor in each animal was completely treated with RF ablation (at $90 \pm 2^\circ\text{C}$ for 3 minutes), and the other remained untreated. Computed tomographic perfusion scans before and after phenylephrine (10 $\mu\text{g/kg}$) or hydralazine (5 mg/kg) administration were performed 2, 7, and 14 days after ablation. Four rats per group were euthanized on each scan day, and pathologic evaluation was performed. The changes of blood flow in the peripheral rim of ablated tumor and untreated viable tumor in response to phenylephrine or hydralazine at each time point were compared. The diagnostic

accuracy of viable tumor using the percentage change of blood flow in response to phenylephrine and hydralazine was compared using receiver-operating characteristic analysis.

Results—The peripheral rim of ablated tumor presented with a hyperemic reaction with dilated vessels and congestion on day 2 after ablation, numerous inflammatory vessels on day 7, and granulation tissue formation on day 14. Phenylephrine significantly decreased the blood flow in the peripheral hyperemic rim of ablated tumor on days 2, 7, and 14 by $16.3 \pm 9.7\%$ ($P = .001$), $24.0 \pm 22.6\%$ ($P = .007$), and $31.1 \pm 25.4\%$ ($P = .045$), respectively. In untreated viable tumor, the change in blood flow after phenylephrine was irregular and insignificant. Hydralazine decreased the blood flow in the peripheral rim of both ablated tumor and untreated viable tumor. Receiver-operating characteristic analysis showed that reliable tumor diagnosis using the percentage change of blood flow in response to phenylephrine was noted on days 2 and 7, for which the areas under the curve were 0.82 (95% confidence interval, 0.64–1.00) and 0.81 (95% confidence interval, 0.56–1.00), respectively. However, tumor diagnosis using the blood flow change in response to hydralazine was unreliable.

Conclusion—Phenylephrine markedly decreased blood flow in the peripheral hyperemic rim of ablated tumor but had little effect on the untreated viable tumor. Computed tomographic perfusion with phenylephrine may be useful in the long-term treatment assessment of RF ablation.

Keywords

Radiofrequency ablation; CT perfusion; vasoactive agents; tumor perfusion; postablation assessment

Interventional oncologic procedures such as chemical ablation, laser ablation, radiofrequency (RF) ablation, and microwave ablation have been widely used in unresectable solid tumor treatment. These techniques can provide efficacious, cost-effective management of localized cancer sites (1,2). However, local recurrence due to factors such as large tumor size (>3 cm), irregular contours, and affluent blood supply continues to be a main cause of treatment failure (3,4). The early detection of residual or locally recurrent tumor after interventional treatment is critical and can facilitate successful retreatment at an early stage.

Contrast-enhanced computed tomography and magnetic resonance (MR) are currently used to evaluate RF ablation efficacy. However, an inflammatory peripheral rim induced by ablation, which presents as enhancement surrounding the coagulated zone, can obscure viable residual tumor. The histologic examination of ablated tumors has shown that the sensitivity of computed tomography or MR for the detection of viable tumor after ablation ranges from 36% to 86% (5–7). Recent reports that positron emission tomography/computed tomography can increase the sensitivity and accuracy for the detection of residual tumor are promising (8,9), but this modality may not be sufficiently cost effective to become a routine follow-up method. New imaging methods are needed for the successful and accurate follow-up of local tumor ablation therapy.

Previous research has shown that tumor vessels are immature, lack normal smooth muscle and pericyte structure, and do not react to vasoactive drugs (10,11). This characteristic has been used for more than two decades to differentiate malignant from benign lesions by angiography, a procedure called pharmacangiography (12). However, angiography is invasive and of low spatial resolution and is seldom used for viable tumor detection after RF ablation. Computed tomographic (CT) perfusion has been recently used to quantify the blood perfusion of a lesion or organ (13–15). This technique requires only an intravenous administration of contrast agent and has excellent spatial and temporal resolution.

We hypothesized that because of the difference between tumor and normal vessels, the perfusion in inflammatory tissue and viable residual tumor surrounding an ablated zone would change differently in response to vasoactive drugs. That is, vasoconstrictors should produce constriction of normal vessels and accordingly decrease blood flow in inflammatory tissue. Vasodilators should dilate normal vessels and increase blood flow in inflammatory tissue. In contrast, tumor blood flow should show no change or behave paradoxically in response to the same vasoactive drugs. Here, the constriction of surrounding normal vessels should “push” blood into the tumor and increase tumor blood flow, while the dilation of surrounding normal vessels should “divert” blood away from tumor and decrease tumor blood flow. This difference could be useful in differentiating viable tumor from the peripheral inflammatory hyperemic rim after RF ablation. Accordingly, the purpose of the present study was to evaluate perfusion changes in viable tumor and the peripheral hyperemic rim of ablated tumor using CT perfusion imaging. A vasoconstrictor, phenylephrine, and a vasodilator, hydralazine, were used to modulate blood flow during a 2-week post-ablation follow-up period.

MATERIALS AND METHODS

Overall Experimental Design

Twenty-four rats with 48 bilateral shoulder tumors were used in this study. The rats were randomly divided into phenylephrine ($n = 12$) and hydralazine ($n = 12$) groups. All right-side tumors were treated with RF ablation. The left-side tumors were untreated and served as internal controls. On days 2, 7, and 14 after RF ablation, animals were examined with CT perfusion scans before and after phenylephrine or after hydralazine. Four animals in each group were euthanized immediately after each scan day. Corresponding tumor slices in orientation to the CT image data were obtained and evaluated with histology. The changes in blood flow in untreated tumors and the peripheral rim of ablated tumors in response to phenylephrine or hydralazine at each time point were compared.

Animal Model

A well-established rat tumor model, DHD/K12/TRb adenocarcinoma in the subcutaneous tissue of BDIX rats, was used in this study (16). The experimental protocols were approved by the Institutional Animal Care and Use Committee at our institution. For all animal experiments, procedures were carried out under general gas anesthesia with 1% isoflurane and an oxygen flow rate of 1 L/min (EZ150 Isoflurane Vaporizer; Euthanex Corporation, Palmer, PA). The DHD/K12/TRb colorectal carcinoma cell line originates from a 1,2-dimethylhydrazine-induced colon adenocarcinoma in BDIX rats (the original source was the European Collection of Cell Cultures). Cells were cultured in RPMI-1640 with 10% fetal bovine serum and passaged weekly. Six-week-old male rats (Charles River Laboratories, Inc, Wilmington, MA) were used for inoculation. Tumors were inoculated subcutaneously with a bilateral injection of 0.05 mL DHD/K12 cell suspension (2×10^6 cells/mL) on the shoulders using a 26-gauge hypodermic needle. Tumor size was measured weekly using calipers. Tumors were grown for 6 weeks, and the maximum diameter was 9.0 to 13.5 mm.

RF Ablation

RF ablation was performed using a 480-kHz RF generator (Radionics, Inc, Burlington, MA) and a custom-designed 21-gauge monopolar needle electrode. Here, the RF antenna and a thermocouple were located in the central core of a disposable, uninsulated hypodermic needle to heat the tissue and monitor temperature during ablation. A grounding pad was placed on the abdomen, and ultrasonic gel was used to ensure proper contact. The skin was cut open about 5 to 7 mm in length with a scalpel to avoid skin burning, and the electrode was inserted into the tumor center to 0.8 cm in depth. During ablation, the temperature was

manually adjusted to $90 \pm 2^\circ\text{C}$ for 3 minutes. The power was approximately 2 to 3 W. This ablation protocol resulted in complete tumor ablation in our preliminary experiments.

CT Perfusion

CT perfusion scans were performed with a 24-slice CT scanner (Sensation Open; Siemens Medical Systems, Erlangen, Germany) 2, 7, and 14 days after RF ablation. The rat position on the CT table was adjusted so that the centers of both tumors were parallel to the scan plane. An initial planar scan was carried out to determine the optimal position for the perfusion scan. Then, the perfusion scan was performed with a 0.5-mL bolus of contrast (Optiray 240; Mallinckrodt, Inc, St Louis, MO) administered through a 24-gauge catheter (Safelet Cath; Exel International Medical Products, Los Angeles, CA) in the tail vein. The perfusion protocol, which imaged 12 consecutive slices every 0.5 seconds for 40 seconds, was executed using the following parameters: axial scan, 80 kV; 150 mAs; rotation time, 0.5 seconds; detector width, 1.2 mm; reconstruction width, 2.4 mm; field of view, 60 mm; and matrix, 512×512 . Thus, each perfusion scan obtained 960 images. Phenylephrine (10 $\mu\text{g}/\text{kg}$; Sigma-Aldrich, Inc, St Louis, MO) or hydralazine (5 mg/kg; MP Bio-Medicals, Inc, Solon, OH) was injected through the tail vein 1 or 5 minutes before the post-perfusion scan. The delay times for phenylephrine and hydralazine were based on the effect-time curves reported in the literature (17,18). The interval between baseline and post-drug perfusion scans was 10 minutes to allow for contrast agent clearance.

Image Analysis

Image analysis was performed by one observer (H.W.) with 5 years of experience in CT diagnosis and 3 years of experience in CT image postprocessing. All preperfusion and post-perfusion studies were analyzed on the Wizard workstation using commercial compartmental analysis-based perfusion software (Body Perfusion CT, Syngo CT 2006A-W Wizard VB20B-W; Siemens Medical Solutions) and a tumor perfusion algorithm. Blood flow was measured in milliliters per 100 milliliters per minute on the basis of the maximum slope method. A single 2.4-mm slice that best depicted both of the bilateral tumors was chosen from the 12 perfusion slices. The analyzed slices of pre- and postper-fusion of each animal were consistent.

CT images of the chosen slice were loaded into the software. The image stack was examined, and images with severe motion artifacts were removed before perfusion calculation. A rectangular reference region of interest (ROI) that contained the tumors was drawn, and two-dimensional motion correction was performed automatically. The arterial input curve of contrast medium concentration was obtained from an ROI (50–70 pixels) in the aorta or common carotid artery. A processing threshold of –50 to 200 Hounsfield units was selected. The time-attenuation curves of artery and mean tissue were generated by the software and displayed in an arterial input function and optimization dialog box. The times of arterial shift and Patlak start for Patlak modeling and the start time, rise time, peak time for maximum slope modeling were adjusted as necessary on the basis of the time-attenuation curves of artery and mean tissue. A grayscale maximum-intensity projection image and pseudocolor perfusion map were generated corresponding to each voxel. ROIs of untreated viable tumor, peripheral rim of ablated tumor, and adjacent normal muscle were drawn on the maximum-intensity projection image. Because only negligible blood flow was found in the center of the untreated tumor and the coagulation zone of the ablated tumor, the enhanced rim of untreated tumor was determined as the untreated viable tumor, and the enhanced rim of the ablated zone was determined as the peripheral rim of the ablated tumor. Care was taken to exclude the surrounding vessels and fat when drawing the tumor boundary. The mean values of blood flow for every ROI were recorded.

Histopathologic Examination

Rats were euthanized with carbon dioxide inhalation immediately after the perfusion scans. The tumors were excised, and the samples corresponding to the CT perfusion scans were obtained and fixed with 10% formalin. The samples were then embedded in paraffin, and 5- μ m slices were obtained and stained with hematoxylin and eosin. All tissue processing was carried out by the Tissue Procurement and Histology Core Facility of the Case Comprehensive Cancer Center (P30 CA43703).

Statistical Analysis

Unless otherwise noted, data are presented as mean $\pm$ standard error. A paired t test was used for the comparison of blood flow before and after drug administration. The differences of predrug blood flow in the peripheral rim of ablated tumor and untreated viable tumor at different time points were compared using unpaired t tests. One-way analysis of variance was used to compare blood flow among three time points. All statistical tests were evaluated by Minitab version 15.0 (Minitab, Inc, State College, PA). A P value $< .05$ indicated a statistically significant difference.

The diagnostic performance for the detection of viable tumor using the percentage change of blood flow in response to phenylephrine or hydralazine was tested with receiver-operating characteristic (ROC) analysis. The detection accuracy of each observation time point and pooled data were measured according to the area under the ROC curve (AUC). ROC curves, AUC values, and 95% confidence intervals (CIs) for each observation time point were analyzed with Analyse-it (Analyse-it Software, Ltd, Leeds, United Kingdom). An AUC of 0.5 indicated that a diagnosis was obtained by guessing.

RESULTS

CT Perfusion Findings and Corresponding Histologic Correlation

The perfusion images and corresponding histology of a typical untreated tumor are shown in Figure 1. The central zone of the tumor exhibited necrosis, and hyperdensity in this zone in the CT image indicates possible calcification (Fig 1a). No perfusion was noted in this area (Fig 1b). In contrast, the outer part of the tumor consisted of viable tumor cells enclosed by a fibrous capsule (Fig 1c). Microvascular morphology was noted in this region and was located primarily in the capsule (Fig 1d). Correspondingly, contrast enhancement and higher perfusion were noted in this area.

After ablation, the skin in the immediate vicinity of the ablated tumor showed swelling and edema on day 2, but by day 7, the swelling and edema had mostly resolved. Although some scar formation was noted on day 14, the ablated tissue appeared fully healed under gross examination. Histology showed extensive cellular damage in the coagulation zone. The peripheral rim of ablated area presented as a hyperemic reaction zone with dilated vessels and congestion on day 2 (Figs 2a and 2b). On day 7, increased vascularization was seen in the peripheral rim, but the lumen of these vessels had returned to normal (Figs 2c and 2d). On day 14, granulation tissue formation and lymphocyte infiltration were seen at the boundary of ablated zone, and the quantity of blood vessels had decreased (Figs 2e and 2f).

The changes in blood flow in the viable tumors of the untreated control and in the peripheral rim of ablated tumor are shown in Figure 3. The blood flow in the untreated tumors was 43.3 ± 11.9 , 50.9 ± 11.0 , and 45.3 ± 9.0 mL/100 mL/min on days 2, 7, and 14, respectively, with no significance differences between the days (analysis of variance, $P = .178$). The blood flow in the peripheral rim of the ablated tumor was greater than that of untreated tumor on days 2, 7, and 14 (49.4 ± 19.5 , 70.3 ± 21.4 , and 69.0 ± 17.2 mL/100 mL/min, respectively).

The differences in blood flow in the peripheral rim of the ablated tumor and the untreated viable tumor were significant on days 7 and 14 ($P = .008$ and $.004$, respectively).

Blood Flow Changes in the Peripheral Rim of Ablated Tumor and Untreated Viable Tumor in Response to Phenylephrine

Table 1 summarizes the blood flow changes in the peripheral rim of the ablated tumor, untreated viable tumor, and muscle in response to phenylephrine during the follow-up period. After the administration of phenylephrine, the blood flow change in the untreated viable tumor was irregular and nonsignificant. The percentage changes were $11.0 \pm 32.7\%$ ($P = .181$), $-4.5 \pm 9.0\%$ ($P = .174$), and $1.8 \pm 35.0\%$ ($P = .483$) on days 2, 7, and 14. In 13 of 24 tested untreated tumors, the tumor blood flow decreased in response to phenylephrine (Fig 4a). In contrast, phenylephrine significantly and consistently decreased the blood flow in the peripheral rim of the ablated tumor. The change rates were $-16.3 \pm 9.7\%$ ($P = .001$), $-24.0 \pm 22.6\%$ ($P = .007$), and $-31.1 \pm 25.4\%$ ($P = .045$) on days 2, 7, and 14. In 23 of 24 tested ablated tumors, blood flow in the peripheral rim decreased in response to phenylephrine (Fig 4b). Figure 5 shows a series of representative CT perfusion images before and after phenylephrine on days 2, 7, and 14. The blood flow in muscle decreased at every time point after phenylephrine administration, but the changes had no statistical significance.

Perfusion Changes in the Peripheral Rim of Ablated Tumor and Untreated Viable Tumor in Response to Hydralazine

The blood flow changes in response to hydralazine in untreated viable tumor and the peripheral rim of ablated tumor are summarized in Table 2. Hydralazine decreased the blood flow in the peripheral rim of both the ablated tumor and untreated viable tumor at every time point, and all changes, except in the peripheral rim of the ablated tumor on day 14, were statistically significant. The decrease was more pronounced in the untreated viable tumor. The change rates were $-27.5 \pm 19.1\%$, $-24.4 \pm 32.1\%$, and $-30.6 \pm 5.1\%$ in the untreated viable tumor and $-15.0 \pm 25.9\%$, $-17.7 \pm 16.8\%$, and $-10.3 \pm 30.7\%$ in the peripheral rim of the ablated tumor on days 2, 7, and 14, respectively. In 22 of 24 tested untreated tumors and 18 of 24 tested ablated tumors, blood flow in the viable tumor or the peripheral rim of the ablated tumor decreased in response to hydralazine (Fig 6). Figure 7 shows representative blood flow images before and after hydralazine administration on days 2, 7, and 14. Hydralazine decreased normal muscle blood flow at every time point, and these decreases were statistically significant on days 2 and 14.

ROC Analysis

ROC curves of the changes of blood flow in response to phenylephrine and hydralazine on days 2, 7, and 14 are shown in Figure 8, and the AUC values and 95% CIs are shown in Table 3. Reliable tumor diagnosis using the percentage change of blood flow in response to phenylephrine was noted on days 2 and 7, for which the AUCs were 0.82 (95% CI, 0.64–1.00) and 0.81 (95% CI, 0.56–1.00), respectively. However, tumor diagnosis using the blood flow change in response to phenylephrine on day 14 and using the percentage change in response to hydralazine was unreliable.

DISCUSSION

The early detection of residual and recurrent tumor in patients undergoing local thermal ablation or chemical ablation is vital. Many imaging modalities, such as computed tomography, MR, and ultrasound are currently used in treatment follow-up to assess tumor response after ablation. In all of these modalities, contrast enhancement is crucial for differentiating the ablated tumor and residual viable tumor on the basis of the fact that the

ablated tumor is void of contrast enhancement, whereas the viable tumor is enhanced because of existing tumor vessels. However, contrast enhancement fails to provide desirable sensitivity (5,6).

The main reason for the low specificity of contrast-enhanced CT or MR examinations in differentiating viable tumor is the physiologic response (nonspecific inflammation) to thermal injury. It is well documented that this response will occur surrounding the ablation zone after ablation therapy. Previous clinical imaging and pathologic correlation studies have shown that this inflammatory rim presents benign periablation enhancement, usually uniform in thickness, and this status will remain 2 to 6 months after ablation. In contrast, viable residual or recurrent tumor has shown focal irregular peripheral enhancement (19,20). However, it is difficult to predict tumor regrowth in the early stage solely on the basis of these morphologic findings. Dromain et al (21) reported that CT and MR imaging may at the earliest depict tumor recurrence at 4 months after RF ablation, because the peripheral rim disappeared with time and was present in only 8% of the RF-ablated areas at this time point.

In our study, the CT perfusion and histologic changes of subcutaneous rat tumor after RF ablation were explored. During the 2-week observation period, the peripheral rim of ablated tumor presented, with a hyperemic reaction with dilated vessels and congestion on day 2, numerous inflammatory vessels on day 7, and granulation tissue formation on day 14. The maximum concentration of blood vessels was observed on day 7. The corresponding CT perfusion study also demonstrated that the blood flow in the peripheral rim increased after RF ablation, was highest on day 7, and had decreased only slightly by day 14. These findings confirm that the inflammatory reaction persists >2 weeks after RF ablation in this animal model, and CT perfusion can successfully monitor blood vessel formation after RF ablation.

Many investigators have used vasoactive drugs to modulate tumor or normal tissue blood flow on the basis of the fact that tumor vessels lack the anatomy necessary to elicit a response following vasoactive drug administration (22,23). In this study, we used vasoactive drugs—phenylephrine as a vasoconstrictor and hydralazine as a vasodilator—to modulate tumor and inflammatory tissue blood flow. Phenylephrine is a powerful post-synaptic α_1 receptor stimulant with little effect on the β receptors of the heart. It produces vasoconstriction that lasts longer than that of epinephrine. It is now the most common over-the-counter decongestant as an oral medicine or as a nasal spray. When used by injection, phenylephrine is used to maintain adequate blood pressure (24). Hydralazine was one of the first available oral antihypertensive drugs and is currently used primarily to treat pregnancy-associated hypertension. It lowers blood pressure by exerting a peripheral vasodilatory effect through a direct relaxation of vascular smooth muscle (25). Both of the agents are commonly used in clinics. They have also been extensively investigated to modulate blood flow in animal experiments (22,26).

We found that phenylephrine significantly and consistently decreased blood flow in peripheral hyperemic rim of the ablated tumor at each time point. This differed considerably from the untreated viable tumor response, which showed no significant changes. These findings are consistent with our hypothesis that the vasoconstrictor phenylephrine can constrict inflammatory vessels, decrease the blood flow in the peripheral rim of tumor after RF ablation, and have little effect on blood flow in the untreated tumor rim. This phenomenon can be helpful to differentiate viable tumor from inflammatory tissue after RF ablation in the early stage.

We also found that hydralazine decreased the blood flow in the peripheral rim of both the ablated tumor and untreated viable tumor. This appears to be opposite to our hypothesis that

a vasodilator would increase inflammatory and normal tissue blood flow but would have little effect on or decrease tumor blood flow. However, with further comparison of the change rates, we found that the response of peripheral hyperemic rim of the ablated tumor was less than that of untreated viable tumor at every time point (-15.0% vs -27.5% on day 2, -17.7% vs -24.4% on day 7, and -10.3% vs -30.6% on day 14). This indicates that the inflammatory vessels surrounding the ablation zone dilated in response to hydralazine, leading to a competing effect and limited blood flow reduction. Therefore, hydralazine decreased the blood flow in the peripheral rim of the ablated tumor to a lesser degree than in untreated viable tumor. The observed changes may also have been a result of hydralazine dose. Significant decreases of normal muscle blood flow on days 2 and 14 indicate that the dose of hydralazine we used was high. A high dose may decrease systemic blood pressure and subsequently reduce blood flow in both ablated and untreated tumor.

ROC analysis confirmed that tumor diagnosis using the change in blood flow in response to phenylephrine was reliable on days 2 and 7. However, tumor diagnosis using the perfusion change in response to hydralazine was not reliable. This further supports the assertion that CT perfusion with phenylephrine might potentially serve as a useful tool in differentiating viable tumor from inflammation. We also found that the test accuracy of perfusion change in response to phenylephrine had no statistical significance on day 14. This may have been due to the limited sample size ($n = 8$). More animals are needed to enhance the statistical power.

A large variability of blood flow in the tumor was noted in this study. For example, the blood flow in the untreated viable tumor ranged from 21.4 to 74.3 mL/100 mL/min. No previous studies have reported the normal range of blood flow in our tumor model. However, we expect that intrinsic factors, such as blood vessel density and blood pressure, and extrinsic factors, such as environment temperature and anesthesia, could all influence baseline flow. Importantly, because we observed the blood flow changes in the same rat and in the same time period (within 10 minutes) in response to phenylephrine or hydralazine, the external factors were minimized, and the observed blood flow changed in response to vasoactive drugs should be reliable.

There were also several limitations to our study. First, we compared the changes in blood flow in response to vasoactive drugs in untreated viable tumor to those in the peripheral rim of completely ablated tumor. This may not strictly represent the clinical scenario, in which the residual viable tumor embeds in the peripheral rim after RF ablation. To discern the residual tumor from the inflammatory tissue in this rat tumor model using clinical computed tomography is difficult because of the limited spatial resolution. The commercially available micro-CT imaging cannot provide sufficient temporal resolution to perform perfusion scans at this stage.

Second, the subcutaneous tumor model used in the present study has some limitations. Our preliminary study showed that the ablated tumor would heal with scab formation in 8 to 14 days after treatment. The scab with ablated tissue would fall off after day 14. This allowed us to follow-up the perfusion changes of the tumor for up to 2 weeks after RF ablation, although a longer period of observation would be critical in a clinical scenario. On the other hand, the pattern of blood supply can be unique in each type of tumor model and in individual organs; hence, we cannot assume that the same outcome will be observed in other tumor models in different organs. Future studies will be performed with liver or kidney tumor models in a larger animal model, such as the rabbit, and follow-up with a longer period to validate the results.

Third, we only tested one vasoconstrictor, phenylephrine, and one vasodilator, hydralazine, and one dose of each agent. More experiments are needed to explore other vasodilators or vasoconstrictors and their corresponding dose response.

Fourth, we evaluated the blood vessels with routine histology (hematoxylin and eosin stain). Although immunohistochemical methods may provide specific information about microvessel density, prior studies and our own unpublished data suggest that blood flow is not highly correlated with microvessel density in similar studies (27).

Many parameters, imaging modalities (eg, MR, ultrasound), and calculation models have been used in perfusion studies (28,29). Other parameters, such as permeability, blood volume, time to peak, and mean transit time have been used to describe tumor perfusion. The changes in blood volume and permeability in response to phenylephrine or hydralazine were investigated as well in this study. However, the difference of the changes of these parameters between viable tumor and peripheral rim of ablated tumor were not significant (data not shown). In terms of perfusion models, deconvolution theory is also widely used. Comparison of the pros and cons of these models and imaging modalities was outside the scope of the present study. However, using different imaging modalities and perfusion models to duplicate this study might aid in validating our hypothesis.

CONCLUSION

We found that perfusion in post-ablation peripheral rim and untreated viable tumors reacts differently to vasoconstrictors, with phenylephrine leading to a marked decrease in blood flow in the peripheral rim of the ablated tumor and little effect on that in the untreated viable tumor. CT perfusion with phenylephrine might potentially serve as a useful tool in the treatment assessment of RF ablation.

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Figure 1.

Correlation of perfusion images ([a] maximum-intensity projection, [b] blood flow) with histology ([c] 4 $\times$, [d] 200 $\times$ [hematoxylin and eosin]) in an untreated tumor. The central zone (*asterisks*) of the tumor presents hyperdense on the maximum-intensity projection image (a), shows no perfusion (b), and appears necrotic on the corresponding histologic image (c). The tumor rim consists of densely packed viable tumor cells encased by a hypervascular fibrous capsule (d).

Figure 2.

Representative hematoxylin and eosin histology ([a,c,e] 4 $\times$, [b,d,f] 200 $\times$) of tumor progression after radiofrequency ablation on days 2 (a,b), 7 (c,d), and 14 (e,f). (a) On day 2, coagulative necrosis was seen in the ablation zone. In the peripheral rim (b) (*white square*), a hyperemic reaction zone with dilated vessels and red blood cell infiltration is present. (c) On day 7, numerous blood vessels were found in the peripheral rim of the ablation zone (d) (*white square*). The lumen of these vessels shrank to normal. (e) On day 14, granulation tissue formed in the margin of the ablation zone (f) (*white square*), and the quantity of blood vessels decreased.

Figure 3. Blood flow in the peripheral rim of ablated tumor (*solid line*) and untreated viable tumor (*dashed line*). Error bar represents the standard error of the mean. * $P < .01$ versus untreated tumor (Student's unpaired t test).

Figure 4.

Plots of percentage change of blood flow in untreated viable tumor (**a**) and peripheral rim of ablated tumor (**b**) induced by phenylephrine to baseline blood flow of 24 tested tumors. SD, standard deviation.

Figure 5. Maximum-intensity projection (MIP) and blood flow images before and after phenylephrine injection on days 2, 7, and 14 show that phenylephrine had little effect on blood flow in untreated tumor (*left*) and decreased flow in the peripheral rim of ablated tumor (*right*). *Dotted lines* mark the region of untreated viable tumor (*left*) and the peripheral rim of ablated tumor (*right*). L, left; RF, radiofrequency.

Figure 6.

Plots of percentage change of blood flow in untreated tumor (**a**) and the peripheral rim of ablated tumor (**b**) induced by hydralazine to baseline blood flow of 24 tested tumors. SD, standard deviation.

Figure 7. Maximum-intensity projection (MIP) and blood flow images before and after hydralazine injection on days 2, 7, and 14 show that hydralazine decreased the blood flow in both untreated viable tumor (*left*) and the peripheral rim of ablated tumor (*right*). *Dotted lines* mark the region of untreated viable tumor (*left*) and peripheral rim of ablated tumor (*right*). L, left; RF, radiofrequency.

Figure 8. Receiver-operating characteristic curves of the percentage change of blood flow in response to phenylephrine (*solid line*) and hydralazine (*dashed line*) on days 2 (**a**), 7 (**b**), and 14 (**c**).

Changes in Blood Flow (mL/100 mL/min) in the Peripheral Rim of Ablated Tumor, Untreated Viable Tumor, and Muscle in Response to Phenylephrine

Table 1

Day	n	Peripheral Rim of Ablated Tumor			Untreated Viable Tumor			Muscle		
		Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change
2	12	52.8 ± 19.5	44.1 ± 18.2 [*]	-16.3 ± 9.7	46.9 ± 12.9	52.2 ± 24.0	11.0 ± 32.7	4.7 ± 3.3	2.9 ± 2.3	-36.8 ± 54.7
2	8	69.2 ± 26.3	52.2 ± 25.1 [*]	-24.0 ± 22.6	51.4 ± 8.2	49.0 ± 8.4	-4.5 ± 9.0	4.8 ± 4.7	3.7 ± 3.7	-1.6 ± 56.8
14	4	73.5 ± 14.5	49.5 ± 17.3 [†]	-31.1 ± 25.4	46.9 ± 10.0	46.5 ± 16.9	1.8 ± 35.0	5.1 ± 5.3	2.1 ± 1.9	-61.2 ± 17.8

^{*} *P* < .05.

[†] *P* < .01 (paired *t* test).

Changes in Blood Flow (mL/100 mL/min) in the Peripheral Rim of Ablated Tumor, Untreated Viable Tumor, and Muscle in Response to Hydralazine

Table 2

Day	n	Peripheral Rim of Ablated Tumor			Untreated Viable Tumor			Muscle		
		Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change
2	12	47.2 ± 20.1	38.1 ± 12.8 [*]	-15.0 ± 25.9	40.9 ± 11.1	28.8 ± 9.1 [†]	-27.5 ± 19.1	5.6 ± 2.3	3.6 ± 2.0 [†]	-23.3 ± 52.7
7	8	71.6 ± 17.0	58.1 ± 14.5 [*]	-17.7 ± 16.8	50.6 ± 13.4	36.2 ± 10.5 [*]	-24.4 ± 32.1	3.4 ± 1.9	2.5 ± 1.7	-16.9 ± 58.5
14	4	64.4 ± 20.6	58.3 ± 31.2	-10.3 ± 30.7	43.7 ± 8.9	30.6 ± 8.7 [†]	-30.6 ± 5.1	6.6 ± 3.1	6.6 ± 3.1	2.5 ± 2.1 [†]

^{*} $P < .05$.

[†] $P < .01$ (paired t test).

Table 3

AUCs and 95% CIs of the Receiver-operating Characteristic Curves of Percentage Changes of Blood Flow in Response to Phenylephrine and Hydralazine

	<u>Phenylephrine</u>		<u>Hydralazine</u>	
	AUC	95% CI	AUC	95% CI
Day 2	0.82	0.64–1.00	0.58	0.33–0.82
Day 7	0.81	0.56–1.00	0.66	0.37–0.95
Day 14	0.81	0.46–1.00	0.75	0.26–1.00
Pooled data	0.83	0.71–0.95	0.63	0.46–0.80

AUC, area under the curve; CI, confidence interval.