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Contrast-Enhanced Computed Tomography and Ultrasound for the Evaluation of Tumor Blood Flow

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Abstract

Objective—We evaluated implanted rat mammary adenocarcinoma tumors during a 5-week period using ultrasound, computed tomography (CT), and histology.

Materials and Methods—Contrast-enhanced ultrasound with a destruction-replenishment imaging scheme was used to derive estimates of blood volume and flow. These ultrasound-derived measures of microvascular physiology were compared with contrast-enhanced CT-derived measures of perfusion and vascular volume made by the Mullani-Gould formula and Patlak analysis, respectively.

Results—The tumor cross-sectional area and necrotic core cross-sectional area determined by the 3 methods were correlated ($r^2 > 0.8$, $P < 0.001$, $n = 15$). The spatial integral of perfusion estimated by CT correlated with the spatial integral of flow from ultrasound ($P < 0.05$). The contrast-enhanced tumor area calculated from the ultrasound analysis was highly correlated with the contrast-enhanced area estimated by CT images ($r^2 = 0.89$, $P < 0.001$, $n = 15$). However, the fraction of the tumor area enhanced by the CT contrast agent was significantly larger than either the fraction enhanced by ultrasound contrast agent or than the viable area as estimated from histology slides.

Conclusion—Destruction-replenishment ultrasound provides valuable information about the spatial distribution of blood flow and vascular volume in tumors and ultrasound analysis compares favorably with a validated contrast-enhanced CT method.

Keywords

destruction-replenishment ultrasound; contrast-enhanced CT; tumor blood flow; perfusion

Angiogenesis, the process by which the body forms new blood vessel networks, is necessary for tumor growth beyond 1–2 mm³ and for tumor metastasis.¹ Judah Folkman has documented the role of the tumor blood supply and nutritive microenvironment in the growth, function, and metastasis of tumors, as well as in tumor cells' susceptibility to external treatments such as radiotherapy.^{2–4} Traditionally, the response to cancer treatment is judged by the reduction in tumor volume.⁵ However, because antiangiogenic agents target the vasculature and not the tumor cells directly, treatment with these agents does not necessarily result in decreasing tumor

size. Thus, noninvasive imaging approaches to study and monitor both the anatomic and functional aspects of tumor vascular physiology are under development and include magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound techniques.^{6–10} In particular, validated imaging methods are being sought to monitor the effects of several new therapies and to serve as surrogate markers of therapeutic response.

Ultrasound Background

Studies using continuous-wave, pulsed-wave, duplex, color, and power Doppler ultrasound methods to examine tumor vasculature have been attempted, with varying results.^{11–14} The development of ultrasound microbubble contrast agents and contrast-specific imaging methods has increased the detectable ultrasound signal from the blood pool by as much as 40 dB. Several groups have developed contrast-specific ultrasound imaging techniques.^{11,15–19} In particular, Verbeek et al²⁰ have demonstrated that the ingress and egress of a contrast agent can be used to estimate the blood flow rate in the kidney and Ragavendra et al²¹ have used a sonographic contrast media to image intraovarian arteries.

Microbubbles oscillate nonlinearly in response to applied ultrasound waves, with a resonant frequency dependent on the bubble diameter and shell composition. Echoes from resonant microbubbles can contain energy at multiples and fractions—“harmonics” and “subharmonics”—of the transmitted ultrasound frequency. Harmonic imaging has been used by Wei et al¹⁸ to assess microvascular cross-sectional area in the myocardium. The same group has also shown that the signal from microbubbles in a canine kidney reflects tissue blood volume fraction.¹⁹ However, during high-pressure wave propagation, nonlinear wave distortion can cause harmonic components to be produced by tissue as well as by microbubbles. Another technique, “subharmonic imaging” uses reception at one half of the transmitted center frequency. Under clinical imaging conditions, tissue echoes do not contain subharmonic components. In the technique described in this paper, subharmonic echoes are generated by transmitting at 6 MHz and receiving at 3 MHz with a broadband 5 MHz transducer. Although the use of a lower receive center frequency may degrade spatial resolution, microbubble detection is improved.

To further reduce tissue echoes, this study uses phase inversion imaging,²² where pairs of pulses are transmitted and the phase is inverted between the pulses in the pair. The sum of the echoes from the 2 pulses will be nearly zero from linear scatterers in tissue, but will be much higher in magnitude from microbubbles, which are highly nonlinear scatterers.

Subharmonic, phase-inversion imaging is combined with a destruction-replenishment imaging method for the estimation of blood flow and volume in the tumor microvasculature.^{22,23} In a destruction-replenishment technique, ultrasound is used first to destroy the contrast agent in a volume defined by the imaging plane and then to nondestructively image replenishment of the agent into that volume. Several other groups have also investigated the use of destruction-replenishment ultrasound imaging. Wei et al¹⁸ first used destruction-replenishment ultrasound to study contrast refill into canine myocardium, and demonstrated that ultrasound-derived measurements correlated with flow estimated with microsphere analysis. Similarly, this study uses a well-mixed tank model to estimate fractional vascular volume and blood flow, $\hat{Q}$, from the signal due to replenishment of contrast agent into an imaging voxel (see Appendix). Koda et al²⁴ have used contrast-enhanced intermittent ultrasound to evaluate the sensitivity of detection of tumor vessels in hepatocellular carcinomas. They found that detection of the tumor vessels was better with this method than with conventional color or power Doppler ultrasound and equal to the rate of detection with dynamic CT for hypo- and iso-echoic nodules.

Previous Results for MRI and CT

In quantifying tissue perfusion and permeability, the other method most commonly used is dynamic contrast-enhanced MRI.^{8,9} This method, however, is not widely available, image acquisition times are longer, and at high doses, nonlinearity exists between the signal enhancement and contrast agent concentration. In contrast-enhanced CT, the concentration of contrast agent in a volume is directly related to the change in signal (measured in Hounsfield Units, HU) from baseline. In the CT literature, Miles et al and Harvey et al have previously applied Patlak analysis to contrast-enhanced CT in normal kidneys and in human prostate tumors and demonstrated that perfusion and permeability [both ml/min/gram of tissue] can be quantified.^{6,7,10,25,26} Patlak analysis involves the calculation of the rate constant of contrast agent uptake by tissue from the blood, in units of contrast concentration in the tissue normalized by the concentration in the blood.²⁷ In estimating tissue perfusion using Patlak analysis, the peak enhancement over time in the tumor is normalized by an arterial input function.²⁷

Study Purpose

In this work, the results of a study are described in which 25 implanted tumors in rats were imaged using a destruction-replenishment ultrasound method and contrast-enhanced CT, with subsequent excision and histologic evaluation. The morphologic, blood volume, and flow rate information that can be obtained using this ultrasound technique are examined in detail, and compared with the information obtained from CT and histology.

METHODS

Study Overview

All protocols were approved by the Institutional Animal Use and Care Administrative Advisory Committee at the University of California, Davis. Cells from a rat mammary adenocarcinoma line were surgically implanted into the dorsal lumbar region of 25 male 300–500 g Fischer 344 rats. After tumor implantation, 5 rats per week were imaged by ultrasound and CT and then killed for histologic examination at the end of weeks 2, 3, 4, 5, and 6.

Ultrasound Imaging

For the imaging studies, each rat was anesthetized using aerosolized isoflurane (Halocarbon Laboratory, River Edge, NJ). The hair over the imaging site was shaved, and a standoff gel pad and imaging gel were placed on the skin surface. A Siemens Elegra ultrasound system with a 5 MHz HDPL40 transducer (Siemens Medical Systems, Issaquah, WA) was used for all imaging modes. A preliminary B-mode examination of the tumor was performed to measure its maximum width, length, and depth, and to locate the largest tumor cross-section in a sagittal or parasagittal plane; the transducer was then fixed in place for tumor destruction-replenishment imaging. B-mode acquisition used a transmission center frequency of 6.5 MHz and a receive frequency of 6 MHz, with a peak negative pressure of 2.2 MPa. The total tumor cross-sectional area and volume from ultrasound was calculated. The volume was calculated using the formula for a prolate ellipsoid

$$\text{Volume}[\text{cm}^3] = \text{length}[\text{cm}] * \text{width}[\text{cm}] * \text{height}[\text{cm}] * \frac{\pi}{6}.$$

A catheter was placed in the tail vein for contrast agent administration. Ultrasound contrast media (Definity, Bristol-Meyers Squibb Medical Imaging, Inc., Billerica, MA) was diluted with physiologic saline to 130 $\mu\text{L}/\text{mL}$ and delivered as a constant rate infusion at a rate of 10 mL/h .

The ultrasound destruction-replenishment imaging technique has been described previously.^{28,29} Briefly, a 5-MHz transducer transmits a customized series of pulses programmed on the Siemens Elegra scanner. The series consists of 5 3-MHz, 2.2-MPa, 5-cycle pulses, which fragment any contrast agent in the imaging plane of the transducer; followed by 29 6 MHz, 800-kPa, 5-cycle phase-inversion pulse pairs used for nondestructive imaging of contrast replenishment of the imaging plane with a receive center frequency of 3 MHz. Because of tissue attenuation, the peak negative pressure within the tumor was estimated to be 400 kPa. Previous in vitro studies have indicated that microbubble destruction at this pressure and frequency does not interfere with the estimation of flow. The relatively low transmission pressure and high center frequency of the nondestructive pulses minimize fragmentation of the contrast agent microbubbles. The imaging pulses are timed to capture both fast and slow replenishment: 9 pulses separated by 100 milliseconds are followed by 10 pulses separated by 500 milliseconds and 10 pulses separated by 1 second for a total replenishment monitoring time of 16 seconds for each cycle.

Destruction-replenishment data were collected from 1 tumor cross-section (in the plane for which the tumor area was maximal) in each rat and for 1 kidney (parasagittal image) in each of 7 of the rats. The complete ultrasound examination required approximately 15 minutes per rat.

CT Imaging

After the ultrasound imaging was performed, each rat underwent contrast-enhanced CT imaging (x/i helical CT scanner, General Electric Co., Milwaukee, WI). Contiguous 1-mm collimated images were obtained through the tumor and surrounding tissue in sagittal or parasagittal planes, as used in the ultrasound examination. The tumor volume [cm³] was calculated by drawing a region of interest around the tumor tissue in each slice (NIH IMAGE, Bethesda, MD) and applying the following equation.

$$\begin{aligned} \text{Volume [cm}^3\text{]} \\ &= (\Sigma \text{ tumor area per slice [cm}^2\text{]} * \text{slice thickness [cm]}) \end{aligned} \quad (1)$$

The slice demonstrating the maximal tumor area (similar to ultrasound) was selected and all additional images were acquired at this site. Images were acquired using a standard (low pass filter) reconstruction algorithm, a 512 × 512 matrix and a 5-cm field of view. Following automated rapid administration of a 1-mL bolus of iodinated contrast media (400 mg I/mL, Conray 400, Mallinckrodt Inc., St. Louis MO), serial static 1-mm images were obtained every 2 seconds for 1 minute. Images were acquired at exposure settings of 120 kVp and 120 mAs with a 2 second image acquisition time.

Histologic Analysis

After CT imaging, each animal was killed and the tumor tissue removed for fixation in a 10% formalin solution. Three 5-μm thick sections equivalent to the ultrasound and CT imaging plane were stained with Hematoxylin and Eosin (H&E) to allow for assessment of the overall tumor morphology. One representative H&E stained section of each tumor was digitized.

Ultrasound Image Processing

The ultrasound imaging sequences were processed (MATLAB, MathWorks Inc., Natick MA^{22,23}) in groups of 3 × 3 pixels (approximately 0.2083 by 0.2083 mm) to obtain the peak enhancement (the maximum signal intensity in a pixel over time), the integrated enhancement (the sum of the signal intensity in a pixel over time), the refill time constant (estimated by

Prony's method), and the flow estimator, $\hat{Q}$ (as described in the Appendix and depicted in Fig. 15).

CT Image Processing

Images obtained with CT were analyzed to estimate perfusion and permeability using the methods of Mullani-Gould and Patlak analysis, as described in Figures 1 and 2.^{25–27,30} The perfusion in each CT voxel is estimated by determining the peak enhancement achieved during the user defined rapid rise portion of the time-intensity curve (typically within the first 30 seconds) and dividing by the integral of the arterial input function to the time at which the peak enhancement is achieved. The arterial input function is calculated as the spatial peak temporal integral over an identified arterial region of interest. The permeability is estimated from the relationship between each voxel's enhancement over time, $C(t)$, and the arterial input function, $B(t)$ such that:

$$\frac{C(t)}{B(t)} = \frac{a \int B(t) dt}{V_r \cdot B(t)} + f_{vv} \quad (2)$$

In both cases, the arterial input function is given by the spatial peak enhancement of a defined arterial region of interest (iliac or femoral artery) over time. In equation 2, a is the blood contrast agent clearance in Hounsfield units (HU) and V_r is the volume of the region of interest: the slope of $\frac{\int B(t) dt}{B(t)}$ versus $\frac{C(t)}{B(t)}$, denoted a/V_r , yields an estimate of permeability. The intercept of this same plot, f_{vv} , is the fractional vascular volume of the region.

Histology Image Processing

The digitized histology images were analyzed using a custom program that allowed interactive definition of tumor regions of interest, and red-level threshold setting to segment regions of viable cells (represented by purple pixels) from necrotic tissue. The total viable area, total tumor area, and fraction of viable tumor (number of purple pixels divided by the total number of pixels in the tumor region of interest) were calculated in each case.

Comparison of CT, Ultrasound, and Histology

Images obtained from ultrasound, CT, and histology were quantitatively compared by employing a program written in a commercially available signal processing package (MATLAB, MathWorks Inc., Natick MA), which allowed the operator (A.R.B.) to draw regions of interest defining the tumor region and the necrotic center of the tumor if one was apparent. This program counted the number of pixels which exceeded an enhancement threshold (15 HU in CT and 32 gray scale units in ultrasound) within the tumor region of interest, and computed the spatial integral over the tumor region of interest (ROI) and the spatial average over enhanced pixels within the ROI of each ultrasound- and CT-derived variable of interest (Fig. 3). Previous experience with this technique has shown that the amplitude thresholds used here adequately define regions of contrast enhancement while eliminating noise. In both ultrasound and CT, the fraction of tumor enhanced was defined as the number of pixels that exceeded the threshold values (referred to as the enhanced area when multiplied by a known mm²/pixel scaling factor) divided by the total number of pixels (or area) within the tumor region.

Statistical Methods

Groups of data were compared using Student's t -tests, and correlations were assessed with Pearson's correlation coefficient, r , and the coefficient of determination, r^2 . Statistical significance was assumed for $P < 0.05$.

RESULTS

Data Collected

Of the 25 rats in the study, 2 were removed from the study and euthanized when the tumors became too large and ulcerated. In the remaining 23 rats, the tumor volumes as measured by precontrast B-mode ultrasound ranged from 303 mm³ to 18 cm³ with an average volume of 5528 mm³. Image data for 22 tumors were obtained with ultrasound (one tumor image file was corrupted and the data was lost) and for 15 tumors with contrast-enhanced CT (CT images were not obtained for 7 rats, and 1 further rat had an inadequate arterial input function because the femoral artery was not located in the imaging plane—data from this rat could not be used for perfusion calculations and therefore were used only in volume and enhanced area analyses); and representative histologic slices were obtained in 21 samples (two histology slides had torn tumor tissue or inadequate staining clarity).

Examples of the ultrasound and CT images for 1 tumor imaged at the end of week 3 are shown in Figure 4. In the ultrasound images, distinct bubble echoes are observed, each contained within the tumor vasculature. In the CT image, the contrast agent leaks out of the tumor vasculature, creating a blurred, continuous pattern of enhancement.

Tumor Morphology

The total tumor volumes measured by B-mode ultrasound and precontrast CT agreed well, with a correlation of $r^2 = 0.98$, $P < 0.001$. The difference between the 2 measurements was not significant according to a paired t test, ($P = 0.09$, $n = 15$).

The tumor cross-sectional areas measured by ultrasound, CT, and histology images also correlated well ($r^2 > 0.8$, $P < 0.001$ for all pairwise comparisons). There were statistically significant differences in the tumor area as measured by CT and ultrasound and by CT and histology ($P < 0.05$, $n = 15$). However, there was not a significant difference between the ultrasound and histology measures of tumor area ($P = 0.28$, $n = 21$). The dimensions of the necrotic center were also highly correlated ($r^2 > 0.9$, $P < 0.001$ for all comparisons) and there were no statistically significant differences in necrotic area between ultrasound and either histology or CT. However, there was a statistically significant difference in necrotic area as measured by CT and histology ($P < 0.05$, $n = 15$).

Analysis of Ultrasound-Derived Variables

Tumor Growth and Development—The tumors in this study grew quickly: 3 of the 5 tumors in the first imaging group, 14 days after implantation, had already developed a significant necrotic center visible by ultrasound and CT. The growth of the tumors between weeks 2 and 6 was therefore smaller than we had hoped since many tumors seemed to have reached a steady-state volume rapidly, but some enlargement was still apparent. The average tumor volume, tumor cross-sectional area, and contrast-enhanced area all increased during the 5-week period studied (Fig. 5). The percentage of perfused tumor decreased during the 5-week period (negative correlations $P < 0.05$). No significant changes in other spatially-averaged measures of flow and vascular volume were found.

Comparison of Tumor and Kidney Variables—The patterns of blood flow in the kidney were significantly different from those in the tumors. The spatial averages of the temporal peak enhancement and $\hat{Q}$ values from ultrasound were calculated in tumors (temporal peak enhancement 20.8 ± 10.0 , $\hat{Q}$ 5.8 ± 2.8) and kidneys (temporal peak enhancement 70.6 ± 6.4 , $\hat{Q}$ 32.8 ± 4.6) from 7 of the rats. Both values were significantly different between the tumor and kidney using a paired t test, rejecting the hypothesis of identical means ($P < 0.01$). The contrast replenishment times were also significantly different ($P < 0.01$): the average tumor

had a replenishment time of 8.1 ± 4.7 seconds (average $\pm$ average within-tumor standard deviation), whereas the kidneys had an average replenishment time of 5.4 seconds with an average within-kidney standard deviation of 3.6 seconds.

Comparison of Ultrasound and CT Analyses

Statistical Comparison of Ultrasound and CT-derived Measures of Flow and Vascular Volume—The enhanced tumor area in the ultrasound analysis was highly correlated with the enhanced tumor area in the contrast-enhanced CT analysis ($n = 15$: ultrasound average $121.3 \pm 61.8 \text{ mm}^2$; CT average $190.0 \pm 93.8 \text{ mm}^2$; $r^2 = 0.89$, $P < 0.001$; Fig. 6). The CT-enhanced area estimates were greater than the ultrasound-enhanced areas, presumably due to the contrast leakage from the vasculature into the tissue interstitium. Similarly, the percentage enhanced area within the tumor ROI was consistently larger in the CT analysis (average 79%; range, 48–100%) than in the ultrasound analysis (average 45%; range, 23–87%) although the 2 measures were correlated with $r^2 = 0.77$, $P < 0.001$. The fraction of viable pixels in ultrasound and CT was also compared with the fraction of viable pixels in histology (Fig. 7). The fraction of CT viable pixels was significantly different from the ultrasound and histology estimates using a paired t test ($P < 0.05$), where the CT estimates (average, 0.79 ± 0.17) were consistently higher than the ultrasound and histology estimates. The fraction of viable pixels in ultrasound (average, 0.45 ± 0.18) and histology (average, 0.48 ± 0.22) were not significantly different ($P = 0.49$).

The spatial integral of perfusion estimated by CT did correlate with the spatial integral of $\hat{Q}$, as well as with the spatial integral of the ultrasound temporal peak enhancement and with the spatial integral of the ultrasound integrated enhancement (all $P < 0.05$; Fig. 8). The spatial average of fractional vascular volume, fvv, derived from the CT analysis did not correlate with any variable from the ultrasound analysis.

Other variables associated with the ultrasound-measured contrast enhancement in the tumors—the spatial integral of integrated enhancement, the spatial integral of the temporal peak enhancement, and the spatial integral of $\hat{Q}$ —all increased with the enhanced area (all correlations $P < 0.05$; Figs. 9 and 10).

Qualitative Comparison of Time-Intensity Curves—Color maps were created of the ultrasound temporal-peak and flow values and the CT perfusion values (Fig. 11). These maps showed similar patterns of enhancement in the 2 Modalities. Although the ultrasound and CT images have not yet been registered to complete a pixel-by-pixel correlation of the perfusion-related variables, similarities were observed between the ultrasound and CT time-intensity curves in small regions within individual tumors as described below.

In a small (303 mm^3) angiogenic tumor where high permeability is anticipated (Fig. 12), the time-intensity curve obtained using ultrasound reaches its peak in less than 10 seconds, although the plot is noisy due to the low signal level. The corresponding CT time intensity curve rises rapidly between 5 and 18 seconds (when the bolus reaches the tumor), and then slowly rises from 18 to 50 seconds, presumably as the agent diffuses into the tissue. The CT-derived permeability estimate for this tumor (0.75 mL/min/g) was among the highest seen throughout the study (mean for all tumors = $0.59 \pm 0.34 \text{ mL/min/g}$). Although not statistically significant due to the heterogeneity within each tumor, the smaller tumors $< 500 \text{ mm}^3$ had higher mean permeability estimates than larger tumors, suggesting they were undergoing the early stages of angiogenesis.

In larger tumors with necrotic centers (Figs. 13 and 14), regions with relatively fast and relatively slow contrast enhancement can be identified. In Figure 13, a region of slow flow can be identified on both the ultrasound and CT parametric images, and the corresponding time-

intensity curves demonstrate the expected slow rise time. In Figure 14, the time-intensity curves for a region containing rapid blood flow (coded pink in the ultrasound image and blue in CT) are provided, and the expected steep slope is demonstrated in each case.

DISCUSSION

Noninvasive, contrast-assisted ultrasound imaging is used for the assessment of tumor microvasculature and takes advantage of the fact that clinical ultrasound systems can produce high-intensity pulses capable of fragmenting microbubble contrast agents, as well as low-intensity pulses capable of detecting and imaging agents with minimal agent destruction. Chomas et al defined the parameters that cause fragmentation of lipid-shelled agents²⁹ and also developed a subharmonic pulse-inversion technique for nondestructive imaging of these agents.²⁸ All microbubbles in one imaging plane can be fragmented and the subsequent contrast agent refill into that plane over time can be observed, obtaining information about blood flow and vascular volume. In an initial study of 11 rats with implanted tumors, it was demonstrated that this method could distinguish the fast, homogeneous blood flow in the kidney cortex from the slower, heterogeneous blood flow characteristic of tumor vasculature.²² This serial study represented a first attempt at implementing the ultrasound method for measuring blood flow in vivo. In another study,²³ this method was used to monitor the changes in tumor blood volume and flow induced by an antiangiogenic drug, and significant differences between the vasculature of treated and control tumors as early as 48 hours after the start of therapy were found. In both of these studies, information obtained from the ultrasound method correlated roughly with information obtained from dynamic contrast-enhanced CT and histology, and the ultrasound method was found to be reproducible.^{22,23} The current study provides a much more extensive comparison between the ultrasound-derived parameters and those that can be obtained from contrast-enhanced CT and histology.

The results of this study show that the spatial extent of tumors and of viable tumor tissue can be measured using contrast-enhanced CT or contrast-enhanced ultrasound, with the results of either analysis correlated with that of postexcision histology. Knowledge of the spatial distribution and volume of blood within a tumor may be essential to understanding the status of the tumor. In addition, these modalities allow functional estimation of tumor blood flow which may be a useful clinical indicator of a tumor's initial susceptibility or response to therapy. Pollard et al have recently shown that contrast-enhanced CT measurements of tumor perfusion and permeability in rat organs are correlated with information obtained from gold-standard methods using fluorescent microspheres and Evans Blue dye, respectively.³⁰ In the current study, further evidence is provided that destruction-replenishment ultrasound can measure variables relevant to blood flow kinetics: we were able to observe changes over 5 weeks of tumor growth in variables related to flow ($\hat{Q}$), vascular volume (temporal peak), and both (integrated enhancement and refill time constant).

The refill time constant, which is proportional to flow and inversely proportional to the fractional vascular volume in an ultrasound voxel, is robust in the presence of attenuation. The spatial average of the refill time constant has been shown to vary significantly across tissues and during tumor development or in response to antiangiogenic therapy.^{23,31} As shown in the examples, the refill time constant for the tumors varies widely across the tumor but did not change significantly with tumor area or with time. The time constant for contrast replenishment, as estimated by ultrasound, can be as high as 10–15 seconds, and volume flow within such regions can also be very low.

The physiologic changes recorded by ultrasound and CT methods change with tumor development over time as anticipated. Specifically, estimates of vascular volume, flow rate, and volume flow increased as the tumors developed. It is well-known that mature tumors

establish a vascular bed with a larger mean vessel size and greater flow velocity³² as shown by this study. Likewise, this particular tumor cell line is notorious for outgrowing its blood supply and developing a necrotic center:³³ a phenomenon clearly identified by CT, ultrasound, and histology. Moreover, as expected, comparison of tumor to renal replenishment times revealed far more rapid flow in the kidney. The adherence of these measurements to the predicted functional characteristics of tumor microcirculation lends credence to their reliability.

Ultrasound estimates of tumor blood flow correlated with the CT-derived spatial integral of tumor blood flow. It is not clear why the ultrasound variables that should be related to fractional vascular volume—the spatial integral and spatial average of peak enhancement—do not correlate with the CT-derived average fractional vascular volume. This may be related to the fact that the ultrasound contrast-enhanced area, which represents that section of the plane that actually contains blood vessels and might be called *vascular* tumor area, is different from the CT contrast-enhanced area, which includes any blood-vessel-containing tissue as well as tissue within the 1–2 mm³ diffusion distance of any blood supply, and represents *viable* tumor area. It was also observed that spatial averages in general do not correlate with tumor size or age, probably because spatial heterogeneity means one “average” value is not representative of the whole tumor.

However, the lack of agreement between the 2 modalities does not come as a complete surprise. The fundamental differences between the types of contrast media, most notably the freely diffusible nature of small molecular iodinated contrast agents compared with the strictly intravascular nature of particulate ultrasound contrast agents, complicates the direct comparison of the parameters derived from these imaging techniques. Although dynamic contrast-enhanced CT derived measures of perfusion and permeability have compared favorably to gold-standard techniques,^{30,34} the inability to discern the exact location of contrast media within a region of interest (ie, intra versus extravascular) has long been a confounding variable. This, in combination with the relatively high ionizing radiation doses associated with dynamic studies (approximately 2 times the radiation dose is delivered during a dynamic study compared with a routine anatomic brain study³⁵) has somewhat limited the use of CT for monitoring clinical patients, particularly in serial studies. We believe this to be one of the main advantages of this ultrasound technique. Because the contrast agent is strictly intravascular, there is no ambiguity in regards to its exact location. For this reason, the measures of vascular volume derived from ultrasound are more accurate than those made from CT. Moreover, the superior temporal resolution, lack of ionizing radiation and relatively low cost make ultrasound an attractive alternative.

CONCLUSION

Noninvasive imaging methods capable of assessing the structure and function of tumor microvasculature would be useful for studying tumor development, assessing tumor status, and monitoring response to therapy. An ultrasound method has been developed that yields information about the vascular volume and flow rate in each voxel as well as the spatial distribution of blood flow within an imaging plane. In this study, results have been compared with those of a validated contrast-enhanced CT method, and it was shown that some comparable information is given by the 2 modalities, particularly about the spatial distribution of flow and flow velocities. Blood vessels are permeable to CT contrast agent, so the CT analysis can also provide permeability information; conversely, the ultrasound contrast agent is confined to the blood pool so ultrasound probably provides a more accurate assessment of microvessel location and fractional vascular volume. Both modalities show promise in the assessment of tumor vasculature: the method to use in a given circumstance will be determined by cost and availability of the imaging method as well as the information desired.

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References

1. Folkman J. Tumor angiogenesis. *Adv Cancer Res* 1985;43:175–203. [PubMed: 2581424]
2. Folkman J. Fundamental concepts of the angiogenic process. *Curr Mol Med* 2003;3:643–651. [PubMed: 14601638]
3. Folkman J, Browder T, Palmblad J. Angiogenesis research: guidelines for translation to clinical application. *Thromb Haemost* 2001;86:23–33. [PubMed: 11487011]
4. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med* 2001;7:987–989. [PubMed: 11533692]
5. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–216. [PubMed: 10655437]
6. Miles KA, Charnsangavej C, Lee FT, et al. Application of CT in the investigation of angiogenesis in oncology. *Acad Radiol* 2000;7:840–850. [PubMed: 11048881]
7. Harvey CJ, Blomley MJ, Dawson P, et al. Functional CT imaging of the acute hyperemic response to radiation therapy of the prostate gland: early experience. *J Comput Assist Tomogr* 2001;25:43–49. [PubMed: 11176292]
8. Gossman A, Helbich TH, Kuriyama N, et al. Dynamic contrast-enhanced magnetic resonance imaging as a surrogate marker of tumor response to anti-angiogenic therapy in a xenograft model of glioblastoma multiforme. *J Magn Reson Imaging* 2002;15:233–240. [PubMed: 11891967]
9. Cohen FM, Kuwatsuru R, Shames DM, et al. Contrast-enhanced magnetic resonance imaging estimation of altered capillary permeability in experimental mammary carcinomas after X-irradiation. *Invest Radiol* 1994;29:970–977. [PubMed: 7890512]
10. Miles KA. Tumour angiogenesis and its relation to contrast enhancement on computed tomography: a review. *Eur J Radiol* 1999;30:198–205. [PubMed: 10452718]
11. Krix M, Kiessling F, Vosseler S, et al. Comparison of intermittent-bolus contrast imaging with conventional power Doppler sonography: quantification of tumour perfusion in small animals. *Ultrasound Med Biol* 2003;29:1093–1103. [PubMed: 12946512]
12. Huber S. Doppler ultrasound in the diagnosis of breast lesions. *Anticancer Res* 1998;18:2147–2150. [PubMed: 9703773]
13. Ferrara KW, Merritt CR, Burns PN, et al. Evaluation of tumor angiogenesis with US: imaging, Doppler, and contrast agents. *Acad Radiol* 2000;7:824–839. [PubMed: 11048880]
14. Wells PT, Halliwell M, Skidmore R, et al. Tumour detection by ultrasonic Doppler blood-flow signals. *Ultrasonics* 1977;15:231–232. [PubMed: 898386]
15. Burns PN. Overview of echo-enhanced vascular ultrasound imaging for clinical diagnosis in neurosonology. *J Neuroimaging* 1997;7(Suppl 1):S2–14. [PubMed: 9128460]
16. Forsberg F, Rawool NM, Merton DA, et al. Contrast enhanced vascular three-dimensional ultrasound imaging. *Ultrasonics* 2002;40:117–122. [PubMed: 12159917]
17. Lucidarme O, Nguyen T, Kono Y, et al. Angiogenesis model for ultrasound contrast research: exploratory study. *Acad Radiol* 2004;11:4–12. [PubMed: 14746396]
18. Wei K, Jayaweera AR, Firoozan S, et al. Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. *Circulation* 1998;97:473–483. [PubMed: 9490243]
19. Wei K, Le E, Bin JP, et al. Quantification of renal blood flow with contrast-enhanced ultrasound. *J Am Coll Cardiol* 2001;37:1135–1140. [PubMed: 11263620]
20. Verbeek XA, Willigers JM, Prinzen FW, et al. High-resolution functional imaging with ultrasound contrast agents based on RF processing in an in vivo kidney experiment. *Ultrasound Med Biol* 2001;27:223–233. [PubMed: 11316531]

21. Ragavendra N, Chen H, Powers JE, et al. Harmonic imaging of porcine intraovarian arteries using sonographic contrast medium: initial findings. *Ultrasound Obstet Gynecol* 1997;9:266–270. [PubMed: 9168579]
22. Chomas JE, Pollard RE, Sadlowski AR, et al. Contrast-enhanced US of microcirculation of superficially implanted tumors in rats. *Radiology* 2003;229:439–446. [PubMed: 14526091]
23. Pollard RE, Sadlowski AR, Bloch SH, et al. Contrast-assisted destruction-replenishment ultrasound for the assessment of tumor microvasculature in a rat model. *Technol Cancer Res Treat* 2002;1:459–470. [PubMed: 12625773]
24. Koda M, Matsunaga Y, Ueki M, et al. Qualitative assessment of tumor vascularity in hepatocellular carcinoma by contrast-enhanced coded ultrasound: comparison with arterial phase of dynamic CT and conventional color/power Doppler ultrasound. *Eur Radiol* 2004;14:1100–1108. [PubMed: 14676975]
25. Miles KA. Measurement of tissue perfusion by dynamic computed tomography. *Br J Radiol* 1991;64:409–412. [PubMed: 2036562]
26. Miles KA, Leggett DA, Bennett GA. CT derived Patlak images of the human kidney. *Br J Radiol* 1999;72:153–158. [PubMed: 10365065]
27. Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 1983;3:1–7. [PubMed: 6822610]
28. Chomas JE, Dayton P, May D, et al. Optimization of non-destructive subharmonic imaging. In: *IEEE Trans Ultrason Ferroelectr Freq Control*, 2000;1993–1942.
29. Chomas JE, Dayton P, Allen J, et al. Mechanisms of contrast agent destruction. *IEEE Trans Ultrason Ferroelectr Freq Control* 2001;48:232–248. [PubMed: 11367791]
30. Pollard RE, Garcia TC, Stieger SM, et al. Quantitative evaluation of perfusion and permeability of peripheral tumors using contrast-enhanced computed tomography. *Invest Radiol* 2004;39:340–349. [PubMed: 15167100]
31. Proceedings of the 2003 IEEE Ultrasonics Symposium. October 2003;1:645–648.
32. Folkman J. The vascularization of tumors. *Sci Am* 1976;234:58–64. [PubMed: 1273568]70–53.
33. Matsuda H, Strebel FR, Kaneko T, et al. Apoptosis and necrosis occurring during different stages of primary and metastatic tumor growth of a rat mammary adenocarcinoma. *Anticancer Res* 1996;16:1117–1121. [PubMed: 8702222]
34. Cenic A, Nabavi DG, Craen RA, et al. Dynamic CT measurement of cerebral blood flow: a validation study. *AJNR Am J Neuroradiol* 1999;20:63–73. [PubMed: 9974059]
35. Publication IICoRPO. Recommendations of the International Commission on Radiological Protection: ICRP Publication 26.
36. Karshafian R, Burns PN, Henkelman MR. Transit time kinetics in ordered and disordered vascular trees. *Phys Med Biol* 2003;48:3225–3237. [PubMed: 14579862]

APPENDIX

Theory

Models of Blood Flow for Microvascular Analysis Using Destruction-Replenishment Methods

The 2 simplest models for the replenishment of contrast agent into an imaging voxel are the plug-flow model and the well-mixed tank model (Fig. 15). Given a vascular volume, V , with a constant flow of blood with contrast concentration, C , into the volume at rate, Q , and assuming zero contrast initially in the volume, the concentration, $c(t)$, within the sample volume is described by

$$c(t) = \begin{cases} 0; & t < 0 \\ t \cdot C \cdot \frac{Q}{V}; & 0 \leq t \leq \frac{V}{Q} \\ C; & t > \frac{V}{Q} \end{cases} \quad (1)$$

for the plug flow model and:

$$c(t) = \begin{cases} 0; & t < 0 \\ C \cdot \left(1 - e^{-t \cdot (Q/V)}\right); & t > 0 \end{cases} \quad (2)$$

for the well-mixed tank model. Further assuming that an ultrasound signal can be detected from a sample volume, V_s , which is proportional to the concentration of contrast agent within the sample volume with constant of proportionality, α , the replenishment signal, $r(t)$, after contrast destruction within the sample volume is described by equations similar to (1) or (2) with the steady-state signal, R , replacing the steady-state concentration, C , where

$$R = \alpha \cdot \frac{V}{V_s} \cdot C \quad (3)$$

and $\frac{V}{V_s}$ is the fractional vascular volume within the sample volume.

In both models, the steady-state signal, R , is proportional to the fractional vascular volume in a given sample volume; the rate of signal return into the sample volume is related to the characteristic refill time, $\frac{V}{Q}$, a function of both vascular volume and flow rate; and the total integral of the signal over time is a function of both the flow rate and the vascular volume.

Simulations by Karshafian et al³⁶ have shown that real microvascular blood flow is probably described by a function intermediate to these 2 models; nevertheless, they provide a good starting point for blood-flow analysis. We find that the well-mixed tank model provides the best fit to our data, and is easy to apply because it does not require a priori knowledge of the refill time constant. The flow rate, Q , in this model can be estimated by the steady-state signal multiplied by the time-constant obtained from an exponential curve fit,

$$\hat{Q}_{\text{mixed}} = S \cdot \frac{Q}{V} = \alpha \cdot C \cdot \frac{1}{V_s} Q \quad (4)$$

FIGURE 1.

Description of CT image analysis. Estimates of perfusion were obtained by implementing the Mullani-Gould equation. First, the peak enhancement in time in the tumor image was found (a). These peak values were then normalized by an arterial input function (b) and perfusion maps were created. Spatial integrals and averages were then calculated from the perfusion maps for comparison with ultrasound volume flow rate estimates.

FIGURE 2.

Description of CT image analysis. Estimates of permeability were obtained by implementing Patlak analysis. In the tumor image, contrast enhancement at time (t) was calculated on a pixel-by-pixel basis (a). The arterial spatial peak was then found at time (t). For each pixel, a plot of $C(t)/B(t)$ versus the temporal integral of the arterial input function divided by the arterial input function was created (c). A permeability estimate was obtained for each pixel by calculating the slope of the line (d). Finally, permeability maps were created.

FIGURE 3.

Description of ultrasound image analysis. Estimates were made on a per-pixel basis using the well-mixed tank model. In the well-mixed tank model, Prony's method was used to estimate the exponential slope term, Q/V . Spatial maps were created of the integrated enhancement, the peak enhancement, and the volume flow rate estimates. These values were then either spatially integrated or averaged for comparison to CT.

FIGURE 4.

Images of contrast agent entering the same tumor in ultrasound (a) and CT (b) at various time points during image acquisition. Although the pattern of enhancement is clearly similar, the difference in contrast agent distribution is noticeable by 12 seconds in to the CT acquisition (see text).

FIGURE 5.

The tumor volume as measured by ultrasound and CT (left panel), and the enhanced tumor area as measured by ultrasound, CT, and histology (right panel) were found to increase during the 5-week study.

FIGURE 6.

Comparison of the area of tumor that enhanced with contrast agent application between ultrasound and CT. The enhanced area was found to be correlated between the 2 modalities ($r^2 = 0.89$, $P < 0.001$).

FIGURE 7.

Comparison of the fraction of viable pixels in histology to the fraction of enhanced pixels in ultrasound and CT. The CT estimates are higher than both the ultrasound and histology estimates, presumably due to the effects of tissue permeability to the CT contrast agent.

FIGURE 8.

This plot compares the spatial integral of the CT perfusion values obtained from the Mullani-Gould method to flow parameters obtained from ultrasound. The volume flow estimates were found to be correlated to the CT values ($r^2 = 0.5202$, $P < 0.01$).

FIGURE 9.

The ultrasound-derived enhanced area in the tumors was compared with the spatial integral of the ultrasound peak enhancement. A significant correlation was found between the ultrasound blood volume estimate and ultrasound enhanced area ($r^2 = 0.9358$, $P < 0.001$).

FIGURE 10.

The ultrasound-derived enhanced area in the tumors was compared with the spatial integral of the ultrasound flow estimate (a). A significant correlation was found between ultrasound spatial integral of flow and enhanced area ($r^2 = 0.8264$, $P < 0.001$). The spatial average over the entire tumor of the ultrasound volume flow rate estimate (b) also showed a significant correlation with the percent viable tumor tissue calculated from ultrasound ($r^2 = 0.7041$, $P < 0.001$).

FIGURE 11.

These images of a large, necrotic tumor demonstrate the comparison of ultrasound volume flow rate (a) to CT perfusion values (c) as obtained using the Mullani-Gould method. The image in (b) is a map of ultrasound temporal peak values.

FIGURE 12.

Gray scale ultrasound image of tumor with overlaid color image of time to 80% contrast replenishment (a-left), and CT image with overlaid color image of time to 80% contrast enhancement (b-left) in a small angiogenic tumor in which high tissue permeability is anticipated. The ultrasound time-intensity curve reaches its peak in less than 10 seconds. The CT time-intensity curve rises most rapidly when the bolus reaches the tumor, between 5 and 18 seconds. As the contrast agent diffuses through the tissue (from 18 to 50 seconds) the curve slowly rises. This tumor had one of the highest CT-derived permeability estimates (0.75 mL/min/g) observed in the study.

FIGURE 13.

Gray scale ultrasound image of tumor with overlaid color image of time to 80% contrast replenishment (a-left), and CT image with overlaid color image of time to 80% contrast enhancement (b-left) with corresponding time intensity curve (right) in a larger tumor with a necrotic center. A relatively slow rise time region outlined. The expected slow rise times are observed in both the ultrasound and CT time-intensity curves.

FIGURE 14.

Gray scale ultrasound image of tumor with overlaid color image of time to 80% contrast replenishment (a-left), and CT image with overlaid color image of time to 80% contrast enhancement (b-left) in the same large, necrotic tumor shown in Figure 13. In this case, a region with fast flow is outlined and both time-intensity curves demonstrate the expected steep, rapid rise. The rapid blood flow is coded pink in the ultrasound image and blue in the CT image.

FIGURE 15.

The plug-flow and well-mixed tank models may be implemented for estimating volume flow rate. The constant of proportionality, α , relates the ultrasound signal, $r(t)$, to the concentration of contrast agent, $c(t)$.