Application of CT in the Investigation of Angiogenesis in Oncology[1]

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TUMOR ANGIOGENESIS: SCIENTIFIC EVIDENCE SUPPORTING THE USE OF COMPUTED TOMOGRAPHY

Tumor angiogenesis is the process by which new blood vessels are formed from the existing vessels in a tumor to promote tumor growth (1). Although angiogenesis is essential for tumor growth and metastasis, tumorigenesis and malignant transformation of the tumor are not dependent on angiogenesis. Angiogenesis is a complex process that is mediated by several angiogenic and antiangiogenic factors produced by the tumor cells, the blood, and the stroma of the host tissue (1–3). The balance in the production of these factors can help predict when angiogenesis will develop; angiogenesis develops when the proangiogenic factors overcome the antiangiogenic factors. The process of angiogenesis includes endothelial proliferation, breakdown of basement membranes of the capillaries, endothelial cell migration into the extravascular space in the stroma, formation of capillary tubes, communication with the postcapillary venules, and blood flow through the new blood vessels (4–7).

Tumor angiogenesis is characterized morphologically by an increase in the number of blood vessels, including new capillaries, capillary sprouts, nonendothelialized capillaries, and arteriovenous shunts (5–9). Some of these vessels malfunction. They tend to be leaky, which allows large molecules such as plasma protein to enter the stroma to form the matrix for new vessels. The new vessels lack smooth muscle and innervation and can be easily compressed. In addition, the blood components within the tumor may also alter the blood flow as they adhere and aggregate to the vessel wall (6). These changes in the tumor vessels make physiologic and hemodynamic changes in the blood flow more complex and heterogeneous among various regions and various types of tumors (8,9). In the regions where tumor growth is active (eg, at the periphery of the tumor) and where new blood vessels develop good communication with veins or arteriovenous shunts, there is evidence that blood flow will increase (6,8,9). In the regions where endothelial cells have high vascular permeability, interstitial pressure will increase and can compress the capillary sprouts and small capillaries (5–9). In those regions, the blood flow could decrease and result in tissue hypoxia and necrosis.

Tumor angiogenesis has important implications in the diagnosis and treatment of various solid tumors. Because angiogenesis is crucial for tumor growth, tumor progression, and tumor metastasis, it can be used as a prognostic indicator to predict the outcome of the disease and treatment (3,10). The morphologic and physiologic changes in tumor blood vessels may also have a major effect on the treatment of various solid tumors, particularly with regard to drug delivery and the effectiveness of radiation therapy (6–9). This is because the delivery of drugs or biologic molecules requires adequate blood flow and capillary permeability to carry the agents to the tumors, whereas radiation therapy would require adequate oxygenation of the tissue. Therefore, the development of clinically applicable techniques that enable the characterization and quantification of tumor angiogenesis would be important in the management of solid tumors. In addition, new treatment strategies have

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been developed to direct the treatment on tumor vascular endothelium or to have a direct effect on angiogenesis (10). These techniques are essential for monitoring treatment.

During the past decade, several techniques have been evaluated in the characterization and quantification of tumor angiogenesis, including microvascular density count (11–14), positron emission tomography (PET) (15), magnetic resonance (MR) imaging (4,16–21), color Doppler ultrasound (US) (22,23), and intravenous contrast material–enhanced computed tomography (CT) (24–29). The results of most clinical studies suggested that tumor angiogenesis as characterized by microvascular density counts obtained by using specific markers for endothelial cells (eg, factor VIII–related antigen, CD31 [platelet–endothelial cell adhesion molecule], and conventional immunoperoxidase staining) showed good correlation with poor prognosis and tumor metastasis (14). Currently, microvascular density count is considered the standard for quantification of angiogenesis in histologic studies. The technique, however, is invasive, which makes it impractical for monitoring treatment, and is limited by random sampling errors and interobserver variability. Imaging techniques have been the subject of extensive investigation for both qualitative and quantitative analysis of tumor angiogenesis.

The fundamental basis for contrast enhancement at CT relies on the exchange of small molecules—iodinated contrast material—between the intravascular space and the extravascular interstitial space after intravenous administration of the contrast material (30–35). Tissue and vascular enhancement can be measured and traced over time. Mathematic models for contrast material exchange have been developed to quantify the tissue blood flow, blood volume, mean transit time, and capillary permeability fraction (30–33,35–39) and will be discussed in the following sections.

TUMOR ANGIOGENESIS: CLINICAL EVIDENCE SUPPORTING THE USE OF FUNCTIONAL CT

The clinical evidence indicating that functional CT techniques can be used as markers of tumor angiogenesis is in three areas: (*a*) the ability to differentiate between benign and malignant lesions (angiogenesis within tumors is reflected by greater enhancement and higher perfusion values); (*b*) the ability to depict occult malignancy (angiogenesis within hepatic metastases results in perfusion changes that are detectable with CT before structural lesions can be visualized); and (*c*) the ability to provide prognostic information (the prognostic information provided with functional CT data parallels that associated with microvessel density). This evidence is elaborated in more detail below.

Benignancy versus Malignancy

The differentiation of cancerous tissue from a benign lesion by using structural criteria is often difficult. An example of this is the relatively common diagnostic problem of the solitary pulmonary nodule. Using a spiral CT system, Swenson et al (26) measured peak enhancement in 107 solitary pulmonary nodules and found that malignant nodules showed greater enhancement than did benign lesions. Furthermore, the degree of enhancement was shown to correlate with the intensity of angiogenesis as determined with histopathologic assessment of microvessel density. Similar results were found in a later study by Zhang and Kono (40), who compared peak enhancement, the tissue-to-aorta enhancement ratio, and perfusion measurements among benign, malignant, and inflammatory solitary pulmonary nodules. For all parameters, malignant and inflammatory nodules had higher values than did benign solitary pulmonary nodules, with the clearest separation provided by perfusion measurements. Inflammatory lesions are also associated with angiogenesis, which would result in similar effects to tumor-associated neovascularization.

The determination of the malignant status of lymph nodes creates a similar diagnostic problem. It has been shown for gastric cancer that malignant nodes enhance more avidly than benign nodes (41), a finding that can be attributed to tumor-associated angiogenesis. Dugdale et al (28) used CT to measure perfusion within lymphoma masses and found that perfusion of less than 0.2 mL/min/mL was indicative of active disease, whereas values greater than 0.5 mL/min/mL were suggestive of intermediate or high-grade lymphoma. Perfusion values decreased with successful treatment.

Occult Malignancy

Because angiogenesis develops in hepatic metastases when they are only 200 μm in diameter, the metastases are potentially detectable from changes in perfusion well before they can be visualized as a discrete mass at conventional structural imaging. The ability of functional CT to demonstrate occult hepatic metastases was demonstrated in three studies. In the first study (42), experimentally induced hepatic micrometastases with a mean diameter of 500 μm were associated with abnormal hepatic perfusion detectable with perfusion CT. In the second study (43), simple measurements of hepatic parenchymal enhancement during dual-phase spiral CT showed that increased arterial phase enhancement in patients with no visible metastases heralded the development of overt lesions within the next 12 months. This increased arterial enhancement could be attributed to

Semiquantitative parameters
- Peak tissue enhancement
- Enhancement rate (initial slope of tissue enhancement curve)
- Time to peak tissue enhancement
- Area of tisue enhancement curve

Absolute physiologic parameters
- Perfusion: arterial perfusion, portal perfusion, perfusion index
- Blood volume
- Mean transit time
- Capillary (or blood-brain barrier) permeability

Quantifiable parameters that can be derived with CT.

the fact that angiogenesis within micrometastases resulted in new vessels derived from the hepatic arterial system with few portal venous connections. In the third study (44), CT perfusion imaging demonstrated that overt liver metastases were associated with an increase in arterial perfusion of more than 0.25 mL/min/mL. Thin-section perfusion images demonstrated that the abnormalities of perfusion were not merely confined to the metastases themselves but extended for some distance into the adjacent liver (44). At pathologic examination, this finding was attributed to angiogenesis within micrometastases adjacent to the overt lesions.

Prognostic Information

There are many pathologic data indicating a relationship between tumor angiogenesis and prognosis, and there is evidence that functional CT can provide comparable information—but in an in vivo manner rather than from biopsy or analysis of a resected specimen. Most pathologic studies have shown a correlation between the areas of highest microvessel density and patient survival. These areas of high microvessel density occur in the zone of angiogenesis, and the high spatial resolution of functional CT makes the technique ideally suited for the visualization and quantification of the physiologic changes within these areas of intense angiogenesis.

In one study of a small group of patients with various primary tumors (27), measurements of arterial perfusion obtained within both metastases and the rim of tissue surrounding hepatic metastases showed a positive correlation with survival (ie, the higher the perfusion measurement, the longer the survival) (27). This correlation is closer for measurements obtained within the peripheral rim of tissue, corresponding to the angiogenesis zone, and is in keeping with the results of pathologic studies. CT perfusion measurements of the liver in patients with colorectal cancer have also demonstrated a relationship between low portal perfusion (<0.25 mL/min/mL) and progressive disease and poor survival (27).

A relationship between CT perfusion measurements and tumor aggression has also been shown for lung cancer (K.A. Miles, unpublished data, 1997). A correlation was found between tumor stage and perfusion measurements (rank correlation coefficient, 0.95), and a statistically significant logarithmic correlation was shown between perfusion measurements at CT and glucose uptake (standard uptake value) at 2-(fluorine-18)fluoro-2-deoxy-D-glucose (FDG) PET (r = 0.85, $P < .04$). In an experimental study involving rabbits with implanted VX2 brain tumor, Fisher et al (29) showed that both blood volume and blood-barrier permeability increase with the age of the tumor. This finding directly demonstrates the usefulness of CT-derived measurements for monitoring the progression of angiogenesis.

FEASIBILITY OF QUANTITATION

The presence of angiogenesis within tumors affects the way in which a tumor mass enhances at CT after the intravenous administration of contrast material. The degree of enhancement is readily quantifiable in Hounsfield units. For typical doses of contrast material, the amount of enhancement is proportional to the concentration of iodine within the region of interest. A series of images obtained at one location over time allows generation of time-attenuation data from which a number of semiquantitative parameters, including enhancement rate (Figure), can be determined. Time-attenuation data also form the basis of dedicated functional CT techniques that allow more accurate quantification of physiologic parameters relevant to the pathophysiology of tumor angiogenesis, specifically perfusion (blood flow per unit volume of tissue), blood volume, mean transit time (average time for blood to traverse the vasculature), and capillary permeability. These functional CT techniques model the distribution of contrast material between the intravascular and extravascular compartments of tissue. A basic modeling assumption is that there is a single source of blood supply or, in the case of multiple supply, that the time profile of the concentration of contrast material in each is the same.

Tracer kinetics modeling of the liver is complicated by the organ's dual blood supply (ie, arterial and portal), which have very different time profiles of contrast material concentration. Angiogenesis within liver tumors results in a

predominantly arterial supply. The portal perfusion of the surrounding liver, however, may be reduced by compression from small tumors, by white cell margination within portal radicles adjacent to hepatic tumors, or by tumor-derived vasoactive substances. The two components of liver enhancement can be quantified separately (eg, by using the time to peak splenic enhancement to divide the liver time-attenuation curve into arterial and portal phases). Thus, specific measurements of hepatic arterial and portal perfusion can be made and the hepatic perfusion index (ie, the ratio of arterial to total liver perfusion) determined.

The quantifiable parameters that can be derived with CT are summarized in the Figure. Automated image analysis that calculates these parameters on a pixel-by-pixel basis can generate parametric images in which quantitative physiologic data are displayed with high spatial resolution. To date, we are aware of no studies that have shown direct correlation of CT measurements of perfusion, permeability, or blood volume with the intensity of angiogenesis at histologic examination (although T.-Y.L. and C.C. are collaborating in one such study). The use of such CT measurements depends on the fact that other techniques have shown these parameters to be altered by the presence of angiogenesis. Histologic assessment of microvessel density, however, was shown to correlate with peak contrast enhancement in a series of 107 solitary pulmonary nodules (26).

Semiquantitative versus Absolute Physiologic Parameters

Peak tissue enhancement is partly determined by blood volume but is also determined by the cardiac output and the dose of contrast material administered (45). Whereas the dose of contrast material can be controlled, cardiac output will vary among patients. Hence, the value of measurements of peak enhancement (and other simple measurements of enhancement) is less than that for absolute CT blood volume measurements, which can be corrected for variations in central circulatory parameters by using enhancement data from the vascular system as an input function. In any individual CT examination, however, comparisons of peak enhancement among different regions will provide an assessment of relative blood volume. A further complication is that simple measurements of contrast material enhancement cannot enable the separate evaluation of intravascular and extravascular components of enhancement. This separation can be achieved only by using the Patlak model (46) or deconvolution-based methods for determining blood volume and capillary permeability (39).

The initial slope of the tissue enhancement curve approximates the product of the tissue blood volume and the initial slope of the arterial enhancement curve. The latter factor is determined by the cardiac output and injection rate; hence, as in the case of peak enhancement, the initial slope is a poor substitute for the absolute measurement of tissue blood volume.

The time to peak tissue enhancement approximates the sum of the time to peak arterial enhancement and half the mean transit time. Unless the arterial enhancement curve is measured and the time to peak tissue enhancement corrected by subtracting the time to peak arterial enhancement, the true mean transit time will be overestimated by an amount dependent on the cardiac output and injection rate.

Validation and Sources of Error

CT perfusion measurements have been validated against microspheres in abdominal organs, normal brain, brain tumors, and skeletal muscle tumors in rabbits (33,38,39; T-Y Lee, et al, unpublished data, 1999) and against thermal clearance techniques and PET in humans (47). Of the two perfusion measurement methods based on the Fick principle (see Methods section), the gradient method is less prone to errors resulting from the washout of contrast material. One of the assumptions of the Fick-based methods is that *none* of the contrast material bolus has left the organ of interest at the time of measurement. This is more likely with the gradient method, as the time of maximal enhancement rate occurs before the time of peak enhancement. The peak enhancement method is less affected by noise. CT perfusion values for normal organs are close to accepted reference ranges. The technique has been shown to be sufficiently sensitive in the measurement of drug-induced changes in tumor perfusion (48) and the reactivities of perfusion to arterial carbon dioxide tension in brain tumors and skeletal muscle tumors (39; T-Y Lee, et al, unpublished data, 1999). Hepatic arterial perfusion measurements correlate well with Doppler measurements of arterial flow, and CT perfusion measurements in lung cancer correlate with glucose uptake as assessed with FDG PET. Validation of CT capillary permeability measurements has proved difficult because the values obtained with different techniques are dependent on the molecular size of the tracer used.

Reproducibility studies have demonstrated a high reproducibility between different operators determining hepatic arterial perfusion from the same data sets (K.A. Miles , unpublished data). The reproducibility of cerebral blood flow and of cerebral blood volume measurements obtained with CT in rabbit brains on the basis of a deconvolution method have been reported to be 13% and 7%, respectively (39).

The main sources of error for all functional CT techniques are photon noise, partial volume effects, and image misregistration owing to patient movement. Photon noise can be minimized by using reconstruction filters with lower cut-off frequencies and by choosing larger regions of interest for measurement, although this will reduce the spatial resolution of the parametric maps. Partial volume effects are more marked for small structures (eg, arteries) and at tissue interfaces (eg, tumor-lung interface). Regions of interest should, therefore, be placed inside the boundaries of the artery or the organ of interest. Cenic et al (38) devised a method for correcting the underestimation of the arterial concentration owing to the partial volume effect. Patient movement effects are usually due to respiratory motion and are greater at the cranial or caudal extremes of an organ. These effects can be avoided by providing patients with clear instructions and by using an appropriate CT section. Certain image processing procedures can also reduce errors due to partial volume effects and motion. For instance, automated thresholding can eliminate from the functional CT image analysis any pixels with initial attenuation values outside a specified range (eg, 0–100 HU), thereby eliminating areas in the image that contain fat or bone. Alternatively, the elimination of any pixels that enhance excessively (eg, more than 100 HU) or demonstrate negative enhancement (eg, decrease by more than 10 HU) will eliminate regions of the image affected by motion.

METHODS

The use of dynamic contrast-enhanced CT to obtain quantitative functional information about tumors has shown a gradual increase in recent years, particularly since the development of continually rotating (spiral) CT systems. A functional CT study can be readily incorporated into existing conventional CT protocols, providing in one examination both structural and functional information that are automatically coregistered. This may obviate nuclear medicine or Doppler US examinations, which would result in greater convenience for the patient and improved cost-effectiveness.

For CT, contrast material is used as the tracer, the concentration of which can be determined from the change in attenuation produced after injection. The relationship between concentration and attenuation change is linear. At 120 kVp, an attenuation increase of 25 HU is approximately equivalent to an iodine concentration of 1 mg/mL, whereas at 80 kVp, an attenuation increase of 32 HU is approximately equivalent to an iodine concentration of 1 mg/mL (T-Y Lee, et al, unpublished data, 1987). Conversely, at the same patient dose, a higher kVp will result in less noise than does a lower kVp. The optimization of the signal-to-noise (contrast-to-noise) ratio at dynamic contrast-enhanced CT studies taking into account patient dose is currently an area of research. Preliminary results indicate that the signal-to-noise ratio is dependent on the size of the object being scanned (W. Huda, personal communication, 1999). For brain studies, initial data suggest that 80 kVp is better than 120 kVp at the same patient dose.

The pharmacokinetics of radiologic contrast material is governed by their distribution in both the intravascular and extravascular extracellular spaces. There is minimal intracellular uptake of contrast material, and excretion is via glomerular filtration. This has allowed three basic data analysis paradigms to emerge: (*a*) model-independent approaches including perfusion measurements based on the Fick principle and deconvolution analysis, (*b*) compartmental modeling as exemplified by the Patlak model, and (*c*) modeling that accounts for convective transport (perfusion) and diffusional exchange (capillary permeability) by means of a distributed parameter model as proposed by Johnson and Wilson (49). In the following section, we will adhere to the system of quantities and symbols proposed by Tofts et al (50). This will unify the description of models in contrast-enhanced CT and MR imaging studies.

Model-independent Approaches

Perfusion measurement methods based on the Fick principle.—The Fick principle asserts that the rate at which the quantity of a tracer Q (in milligrams) will accumulate in an organ at any time t is dependent on the organ blood flow F (in milliliters per minute) and the concentration of the tracer in the artery and veins, $C_a(t)$ and $C_v(t)$, respectively (in milligrams per milliliter), as follows:

$$\frac{dQ(t)}{dt} = F\left[C_a(t) - C_V(t)\right] . \qquad (1)$$

CT provides the opportunity to measure regional (local) blood flow within a tissue or organ. In this case, the Fick principle as stated in Equation (1) must be reinterpreted as follows: $Q(t)$ is the mass concentration of contrast material in tissue (in milligrams per gram) and F is the specific blood flow (in milliliters per minute per gram); $C_a(t)$ and $C_v(t)$ remain the arterial and venous concentration (in milligrams per milliliter).

After the injection of a bolus of contrast material, there will be a short time within which the bolus is wholly retained

within the organ. For this period, the venous concentration $C_v(t)$ will be zero. Thus,

$$\frac{dQ(t)}{dt} = FC_a(t) . \quad (2)$$

The rate of tracer accumulation will be maximal when the arterial concentration is maximal, as follows:

$$\left[\frac{dQ(t)}{dt}\right]_{Max} = \left[FC_a(t)\right]_{Max} . \quad (3)$$

Because the attenuation change within the organ of interest gives volume tissue concentration Q_v rather than mass concentration $Q(t)$, Equation (3) is multiplied by tissue density to give

$$\left[\frac{dQ_v(t)}{dt}\right]_{Max} = \rho\left[\frac{dQ(t)}{dt}\right]_{Max} = F\rho\left[C_a(t)\right]_{Max} . \quad (4)$$

The difference between Equations (3) and (4) is in the presence of tissue density as the scaling factor. Because ρ is close to unity in most cases, it will be neglected to simplify the notation in the following. From a rapid series of CT images, $[dQ(t)/dt]_{Max}$ can be determined from the maximal slope of a tissue time-attenuation curve and $[C_a(t)]_{Max}$ from the maximal attenuation within an artery, typically the aorta. Hence, with the gradient method, the rate of perfusion is obtained by dividing the maximum rate of tissue enhancement by the peak aortic enhancement.

An alternative algorithm, the peak enhancement method, has been proposed for CT and uses the integral of Equation (4), as follows:

$$\left[Q(t)\right]_{t=T_{Max}} = F\int_0^{T_{Max}} C_a(t)dt , \quad (5)$$

where T_{Max} is the time at which peak tissue enhancement occurs. Although less sensitive to noise, this algorithm tends to provide an underestimation of perfusion. This is because the time point of measurement is later (ie, peak tissue enhancement occurs after $[dQ(t)/dt]_{Max}$), and typically a fraction of the bolus has left the organ of interest, negating one of the assumptions of the technique.

A typical image acquisition sequence for perfusion CT based on the Fick principle lasts about 50 seconds and comprises 1-second images obtained without table movement every 3–5 seconds after injection of a 25.0–50.0-mL bolus of conventional intravenous contrast material at a rate of 10 mL/sec. Perfusion values can be calculated manually or by using automated image analysis such as that incorporated in the Winfun software developed at the Department of Radiology, Cambridge University, Cambridge, England.

Deconvolution method.—When a unit quantity of contrast material is injected as a bolus into an arterial input and the mass of contrast material remaining in a given vascular network over time is measured with a CT scanner, an impulse residue function (IRF) is obtained (51). If the blood flow is constant and the concentration of contrast material in the tissue is linearly dependent on the input arterial concentration, then, according to the principle of linear superimposition, it can be shown that the tissue enhancement curve is related to both the arterial enhancement curve and the IRF, as follows:

$$Q(t) = F\cdot\left[C_a(t)\otimes R(t)\right] = C_a(t)\otimes F\cdot R(t) , \quad (6)$$

where $R(t)$ is the IRF and ⊗ is the convolution operator. The importance of the flow-scaled IRF, $F \cdot R(t)$, is that when the contrast material does not leak across the capillary endothelium (eg, in the brain when the blood-brain barrier is intact), the area and the maximum height of the flow-scaled IRF is the blood volume and blood flow, respectively, of the tissue region under consideration, and the ratio of area to maximum height is the mean transit time (53). Cenic et al (38) demonstrated the feasibility of calculating the flow-scaled IRF from deconvolution of $Q(t)$ and $C_a(t)$ measured with CT, and the blood flow determinations have been validated against microsphere measurements in the brains of normal rabbits. Cenic et al (39) further extended the deconvolution method for the case when the capillary endothelium is permeable to contrast material. The extended method was applied to brain tumors in rabbits to measure blood flow, blood volume, mean transit time, and capillary permeability simultaneously from a single CT study. The CT blood flow measurement was validated against microsphere measurements (39). In the same study, Cenic et al also calculated the precision of CT in the determination of cerebral blood flow and cerebral blood volume to be 13% and 7%, respectively. Essentially the same method was applied by Singal et al (53) to measure renal blood flow and the extraction efficiency of contrast material by the kidneys. Finally, perfusion measurements in skeletal muscle tumors in rabbits were compared with microsphere measurements and found to be accurate. The precision of CT perfusion measurements in this study was also about 13% (T-Y Lee, et al, unpublished data, 1999).

A typical image acquisition sequence for perfusion CT based on deconvolution lasts about 40 seconds and comprises 1-second images obtained without table movement every second after injection of a 40-mL bolus of conventional intravenous contrast material at a rate of 1– 4 mL/sec.

Compartmental Modeling for CT Permeability Imaging

The theoretical basis of CT permeability imaging is derived from Patlak analysis, a nuclear medicine data processing technique for determining the rate constant of tissue uptake of a tracer from the vascular space by using values of tracer concentration in tissue and blood. Patlak analysis makes three basic assumptions. First, the analysis assumes that the pharmacokinetics of the injected indicator material conform to a two-compartment model. Second, the analysis assumes well-mixed compartments. Finally, the analysis assumes that the blood hematocrit levels within major vessels and capillaries are equal.

After contrast material is injected into the intravascular space, it will pass into the extracellular space at a rate that can be defined by a rate constant K^{trans}. There is also exchange from the extracellular into the intravascular space with a rate constant K^{trans}/v_e, where v_e is the extravascular distribution space of contrast material; however, for the initial period following the bolus injection—typically 2 minutes—the amount of contrast material passing from the extravascular to the intravascular space will be negligible.

At any moment, a volume element (or voxel) of tissue will contain both intravascular and extracellular contrast material. This can be expressed mathematically as follows:

$$Q(t) = V_b \cdot C_a(t) + K^{trans} \cdot \int_0^t C_a(u)du\,, \qquad (7)$$

where $Q(t)$ is the total concentration of contrast material in the tissue at time t, $C_a(t)$ is the concentration of contrast material in the arteries at time t, and v_b is the blood volume in the tissue. In Equation (7), the first term expresses the intravascular component of enhancement and the second term describes the extravascular component.

The Patlak maneuver divides both sides of Equation (7) by $C_a(t)$. Hence,

$$\frac{Q(t)}{C_a(t)} = V_b + K^{trans} \cdot \frac{\int_0^t C_a(u)du}{C_a(t)}\,. \qquad (8)$$

The Patlak plot displays $Q(t)/C_a(t)$ on the y axis and $[\int_0^t C_a(u)du]/C_a(t)$ on the x axis, yielding a straight line with slope K^{trans} and intercept v_b. The "best fit" for the straight line portion can be determined by using regression analysis, with the correlation coefficient reflecting the quality of the curve-fitting procedure. After an initial period of approximately 2 minutes, the plot no longer remains linear as substantial reverse transfer of contrast material from the extracellular to the intravascular space occurs.

To avoid making the assumption that the back flux of contrast material from the extravascular space to the intravascular space is negligible, Yeung et al (24) implemented the full solution of the Patlak two-compartment model,

$$Q(t) = V_b \cdot C_a(t) + K^{trans} \cdot \int_0^t C_a(u)\, e^{-\frac{K^{trans}}{v_e}(t-u)} du\,, \qquad (9)$$

and used nonlinear regression techniques to derive K^{trans} and v_b maps in the brain tumors, demonstrating that steroids decrease the K^{trans} in brain tumors.

A typical image sequence for CT permeability imaging is slower than that used for perfusion imaging (46). One-second image acquisitions are performed without table movement every 10 seconds for 2 minutes. Repeated volume acquisitions would be possible with modern spiral CT systems but with increased radiation burden. Automated image analysis on a pixel-by-pixel basis enables functional images to be generated. A map of blood volume can be generated by plotting the intercept value for each pixel; plotting the slope creates a map of capillary permeability. Compared with the nonlinear regression method of Yeung et al (24), the Patlak analysis has the advantage of being simple and, hence, efficient in the calculation of parametric maps of K^{trans}. The assumption that back flux from the extravascular into the intravascular space can be neglected during early times after injection, however, will depend on the relative magnitude of blood flow and the capillary permeability surface area product (PS) (55). For the case where PS is much greater than F (ie, the extraction ratio $[E]$ approaches 1), it may not be correct. A more fundamental drawback of compartmental models is also revealed under this condition. When the extraction ratio is high, it is expected that the concentration of contrast material in the arteries will be higher than that in the veins, so that the intravascular space can no longer be regarded as a compartment. Specifically, there are three regimens for the Patlak two-compartment model: (*a*) When PS is much less than F, K^{trans} approximates PS; (*b*) when PS is equal to F, K^{trans} is equal to the product of F and E so that separate determination of F and E is not possible; and (*c*) when PS is much greater than F, so that E approaches 1, K^{trans} is equal to F. Even in this last regimen, however, the finite transit time of contrast material through the capillaries would lead to an inaccurate determination of blood flow for tracers with the two-compartment model or the more traditional Kety model (37).

Distributed Parameter Models

A general model that applies under all values of PS relative to *F* was first proposed by Johnson and Wilson (47). This model incorporates a concentration gradient within the intravascular space (capillary) from the arterial inlet to the venous outlet while the extravascular space is modeled as a compartment. The complexity of the solution, being in the Laplace domain, has hindered its acceptance into general use since its first derivation. St Lawrence and Lee (36) discovered a simple analytical solution of the model in the time domain involving blood flow, blood volume, mean transit time, and capillary permeability as the parameters of the solution. Although the use of this simplified solution has not been reported in CT studies, Henderson et al (55) have successfully applied the model to contrast-enhanced MR imaging to study spontaneous canine breast tumors. Similar to the deconvolution method, the advantage of this simplified solution of the Johnson and Wilson model is that it allows the simultaneous determination of four physiologic parameters—blood flow, blood volume, mean transit time, and capillary permeability—from a single CT or MR study. In this respect, both methods are extremely versatile in the study of tumors in which one would expect a wide range of PS values relative to *F*. Whereas the whole IRF must be estimated nonparametrically in the deconvolution method, only four parameters must be estimated in the simplified solution of the Johnson and Wilson model, making the latter more computationally efficient.

A typical image acquisition sequence for functional CT studies based on the distributed parameter model is a two-phase study. The first or dynamic phase is similar to that for deconvolution-based methods: 1-second images are obtained without table movement every second for 40–60 seconds immediately after the injection of contrast material. The second or late phase follows the dynamic phase and is similar to that for the compartmental modeling method: a 1-second image is acquired every 10 seconds for 2 minutes after the first phase.

CURRENT STATUS OF CT REGARDING ANTIANGIOGENESIS

CT is currently widely used in clinical practice and research to demonstrate changes in tumor size after therapy. Functional CT techniques can be readily incorporated into existing CT protocols, thereby providing structural imaging and functional information relevant to angiogenesis or antiangiogenesis therapy within one examination. Unlike other techniques, functional CT offers absolute measurements of capillary perfusion and permeability, quantified in physiologic terms, along with the ability to generate parametric images with high spatial resolution.

To our knowledge, no studies to date have assessed the ability of functional CT techniques to demonstrate changes in perfusion, blood volume, or capillary permeability after antiangiogenesis therapy. The ability of CT to measure changes in tumor perfusion in response to drug therapy, however, has been reported (48). One study (48) demonstrated a reduction in the perfusion of colon cancer metastases in response to BW12C, an agent intended to render tumors hypoxic. The reduction in perfusion was greater for tumors with higher baseline perfusion, reflecting the effects of increased drug delivery. Another study (56) used CT blood-brain barrier imaging to demonstrate an increase in tumor permeability in response to a bradykinin analog, RMP-7.

COMPARISON WITH OTHER TECHNIQUES

Functional CT techniques enable the quantification of perfusion, blood volume, and permeability at the capillary level and, therefore, reflect tumor angiogenesis more directly than do measurements of blood flow within the vascular supply to tumor-bearing organs (eg, those obtained with Doppler US). The spatial resolution of functional CT is greater than that with nuclear medicine techniques, including PET. Spatial resolution is important for differentiating regions of maximal angiogenesis from necrotic areas, a distinction that may otherwise be lost owing to partial volume effects. The linear relationship between iodine concentration and measured attenuation at CT has facilitated measurement of the high vascular concentrations of contrast material that occur, especially during first-pass studies. The nonlinear relationship between concentration of gadolinium-based contrast agent and signal intensity, combined with the presence of velocity-induced signal intensity changes in large vessels, has created substantial difficulties in obtaining vascular time-intensity data with MR imaging. Consequently, many MR imaging studies have used tissue time-intensity curves alone, resulting in substantial errors due to an inability to correct for interpatient variations in cardiac output and central blood volume. Gadolinium-enhanced MR studies are also hampered by relaxivity effects, which do not occur with the iodinated contrast media used for functional CT. When gadolinium-based contrast agent is intravascular, tissue water molecules will be too far from the gadolinium to be affected by its paramagnetic properties. When gadolinium-based contrast agent is within the

extracellular space, however, it is distributed more evenly, and thus all water molecules will be affected. Hence, as gadolinium-based contrast agent passes from an intravascular to an extracellular space, the amount of signal change for a given concentration of the agent (relaxivity) will increase. To date, no correction has been proposed for this phenomenon. This problem does not occur with functional CT because iodine alters CT signal directly, rather than by means of an indirect paramagnetic mechanism.

DEVELOPMENT OF NEW CONTRAST MEDIA

The published methods that use CT to obtain quantitative information with regard to tissue perfusion, vascular volume, and capillary permeability are based on the use of low-molecular-weight, water-soluble contrast material. These media have intrinsic limitations for the purpose of measuring vascular volume due to a rapid transfer of contrast material from the intravascular to the extravascular space. This transfer can be mathematically modeled (and minimized by changing injection parameters), but all of these methods have inherent limitations owing to the unpredictability of many factors in biologic systems (eg, arteriovenous shunts and nonuniform tissue with necrosis, fibrosis, or hemorrhage). A further complicating factor is that, unlike MR imaging or nuclear medicine techniques, CT uses a relatively large amount of iodine to increase attenuation values in tissue. Most of the tumors for which knowledge of the status of angiogenesis would be desirable are relatively hypovascular (eg, colon cancer, lung cancer, pancreatic cancer, some breast cancers). As a result, only small increases in tumor attenuation are seen after the injection of contrast material. Thus, photon noise will be a proportionately larger source of error with hypovascular tumors than with hypervascular tumors.

A one-compartment contrast material with a long vascular residence time would solve many of the theoretical problems encountered with low-molecular-weight, water-soluble contrast material. The contrast material could be injected intravenously, and the increase in tumor attenuation at a fixed time could be used to calculate the intravascular iodine concentration within the tumor. This iodine concentration should directly reflect the tumor vascular volume, with no contribution from contrast material in the extravascular space. The attenuation value should then be a reproducible parameter that can be used serially to follow the effects of treatment on the overall vascular volume. There are many precedents for this type of analysis; however, they have been almost exclusively explored with MR imaging and macromolecular contrast material. The theoretical limitations of MR contrast material are dealt with in a separate section. CT blood pool agents have been investigated, but, to our knowledge, none are currently undergoing clinical trials. Most of the CT blood pool literature has focused on the possibility of increased characterization of liver tumors or vascular imaging before the development of helical CT technology and has not dealt with the possibility of quantification of vascular volume.

Another area in which high-molecular-weight contrast material can be useful is in the measurement of capillary permeability. Capillaries in benign and malignant tumors may have different permeabilities to high-molecular-weight contrast material but similar permeabilities to low-molecular-weight material. Thus, measurement of capillary permeability to high-molecular-weight contrast material may provide a simple in vivo test for malignancy.

RECOMMENDATIONS

Functional CT techniques have been validated as tools for measuring various physiologic parameters within human tumors. Further studies, however, are necessary to validate their use as markers for the efficacy of antiangiogenesis therapy. Specifically, a correlation between histopathologic features of angiogenesis (eg, microvessel density, expression of vascular endothelial growth factors) and imaging parameters for functional CT must be established for a variety of tumors. Data should also be obtained to demonstrate the changes in functional CT parameters resulting from antiangiogenesis therapy. Such data could be readily obtained by including functional CT into research protocols that currently use conventional CT to monitor the morphologic effects of antiangiogenesis treatments.

One important advantage of CT is that it can be used to study almost all tumors in the human body. On the basis of our experience, the following is a guide to the application of functional CT in tumor imaging: For primary tumors of the lung, pancreas, and kidney and for lymphoma, functional CT should be performed to evaluate perfusion, blood volume, and mean transit time. For primary tumors of the liver, functional CT should be performed to evaluate perfusion. For primary tumors of the brain, functional CT should be performed to evaluate capillary permeability. For secondary tumors of the lung, mediastinum, abdomen, and pelvis and for superficial metastases, functional CT should be performed to evaluate perfusion, blood volume, and mean transit time. For secondary tumors of the liver, functional CT should be performed to evaluate perfusion.

Specific recommendations in the development of CT functional imaging in angiogenesis are as follows: (*a*) Techniques should be developed so that more than one functional parametric map can be derived from a single study. (*b*) The optimization of contrast-to-noise ratio with respect to patient dose should be investigated more fully. (*c*) The expected heterogeneity of tumor physiology would argue strongly for the use of multisection scanners so that at least 2 cm can be covered in the axial direction. For cases where more than 2 cm of coverage is required, an additional study with a second injection of contrast material is warranted. The second study can be delayed by as little as 10 minutes from the first study. (*d*) The strength, volume, and injection rate of contrast material must be tailored to the analysis method employed. The method based on the Fick principle requires injection rates of more than 10 mL/sec, whereas the deconvolution-based method and model-dependent methods—namely, the two-compartment Patlak model and the distributed parameter model—require a lower injection rate (ie, approximately 3–4 mL/sec). (*e*) The framing rate, which determines the patient dose if the technique parameters for each image remain the same, is dependent on the parametric map required. The framing rate should be 1–3 seconds per image to obtain parametric maps of blood flow and mean transit time, 1–5 seconds per image to obtain a parametric map of blood volume, and 1–10 seconds per image to obtain a parametric map of capillary permeability. (*f*) The development for clinical use of CT contrast media with long vascular residence times or larger molecular weights would not only improve the measurement of permeability but also that of perfusion, blood volume, and transit time.

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