Published OnlineFirst February 10, 2009; DOI: 10.1158/1535-7163.MCT-08-1016

Blood flow and V_d (water): both biomarkers required for interpreting the effects of vascular targeting agents on tumor and normal tissue

Barbara Kötz, Catharine West, Azeem Saleem, Terry Jones, and Patricia Price

Cancer Research UK PET Oncology Group, Academic Department of Radiation Oncology, The University of Manchester, Manchester, United Kingdom

Abstract

Positron emission tomography studies with oxygen-15–labeled water provide *in vivo* quantitative tissue perfusion variables—blood flow and fractional volume of distribution of water [V_d (water)]. To investigate the relationship between perfusion variables and the effect of vascular-targeting agents on vasculature, we measured tissue perfusion in tumors, spleen, kidney, and liver before and after treatment with combretastatin-A4-phosphate, a combination of nicotinamide and carbogen (N/C), and interferon (IFN). We observed that mean tumor blood flow and V_d (water) was lower than in kidney, liver, and spleen at baseline. Blood flow and V_d (water) were related in tumor ($r = 0.62$; $P = 0.004$) at baseline, but not in other normal tissues evaluated, where minimal variations in V_d (water) were observed over a wide range of blood flow. Despite the relationship between blood flow and V_d (water) in tumors before intervention, vascular-targeting agent–induced changes in these perfusion variables were not correlated. In contrast, changes in blood flow and V_d (water) correlated in kidney and spleen after N/C and in kidney after combretastatin-A4-phosphate. The close relation between blood flow and V_d (water) in tumors but not normal tissue may reflect barriers to fluid exchange in tumors because of necrosis and/or increased interstitial fluid pressure and underlies the importance and interdependence of these positron emission tomography perfusion variables under these conditions. As blood flow and V_d (water) signify different aspects of tissue perfusion, the differential effects of interventions on both variables, flow and V_d (water), should therefore be reported in future studies. [Mol Cancer Ther 2009;8(2):303–9]

Received 10/27/08; accepted 12/17/08; published OnlineFirst 02/10/2009.

Grant support: Medical Research Council Funding of the Medical Research Council Cyclotron Unit at Hammersmith, London, and Cancer Research UK Program grants (C153/A4331 and C153/A1797).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Pat Price, Academic Department of Radiation Oncology, The University of Manchester, Christie Hospital NHS Foundation Trust, Wilmslow Road, Manchester M20 4 BX. UK. Phone: 44-161-446-8110; Fax: 44-161-446-8111. E-mail: pprice@manchester.ac.uk

doi:10.1158/1535-7163.MCT-08-1016

Introduction

Vascular targeting is an effective antitumor strategy that includes a number of different approaches (1). Damaging established blood vessels in tumors can rapidly shutdown perfusion, leading to extensive ischemic necrosis (2, 3). Inhibiting new blood vessel formation can cause tumor growth arrest and apoptosis (4). Conversely, improvement of tumor perfusion may reduce hypoxia, enhance the effects of radiotherapy, and increase the delivery of chemotherapy and molecular-targeted therapies to cancer cells (5). Faced with a growing number of such agents entering clinical trials, there is a recognized need for appropriate end points to assess their clinical efficacy (6). These end points would not only serve as surrogate markers of response in early clinical trials but also improve our understanding of mechanisms of action. To exhibit therapeutic benefit, tumor selectivity is required, making it essential to assess changes not only in tumor but also normal tissue.

There is an increasing interest in evaluating imaging approaches, in particular magnetic resonance imaging and positron emission tomography (PET), to monitor the efficacy of vascular targeting agents (7, 8). PET with oxygen-15 (^{15}O)-radiolabeled water as a tracer to measure regional blood flow is an established technique in the pharmacodynamic evaluation of such agents (9). The method also allows determination of the tissue/plasma partition coefficient of water (i.e., the measured concentration of tracer agent in tissue relative to that in plasma), which in the PET literature is generally called the volume of distribution of water [V_d (water); refs. 6, 10]. As a measure of the amount of ^{15}O-labeled water in a fraction of tissue, V_d (water) presumably reflects the amount of viable tissue per unit volume measured and may also reflect barriers to fluid exchange such as raised interstitial fluid pressure (IFP; ref. 11).

Previous work by us used PET with ^{15}O-labeled water to study the hemodynamic effects of a variety of vascular targeting agents (8, 12–14). A rapid decrease in tumor blood flow was seen after administration of the vascular disrupting agent combretastatin-A4-phosphate (CA4P), which targets established blood vessels lined by immature endothelial cells (8, 15). IFN is thought to have direct and indirect antiangiogenic properties: the former by inhibiting endothelial-cell motility and the later by inhibiting tumor cell expression of basic fibroblast growth factor, a

Published OnlineFirst February 10, 2009; DOI: 10.1158/1535-7163.MCT-08-1016

proangiogenic protein (4). Treatment with IFN for 1 week led to some increase in tumor blood flow, which has recently been attributed to improvements in vessel structure and function (16). An increase in tumor blood flow was also seen with the combination of nicotinamide and carbogen (N/C; ref. 13).

In this article, we review and report our findings obtained from previous PET studies in patients before and after intervention with different vascular targeting agents. We examined their effects on normal tissue and tumor perfusion with particular emphasis on the relationships between blood flow and V_d (water). We hypothesized that V_d (water) is an independent measure of tissue perfusion giving valuable information on tissue hemodynamics.

Patients and Methods

Measurements of blood flow and V_d (water) from three previous PET perfusion studies were used (8, 13, 14). Patients who had at least one type of normal tissue evaluated in addition to tumor lesions were included in this analysis. For all studies, ethical approval had been obtained from Hammersmith and Charing Cross Hospital Research Ethics Committees and permission to administer radioisotopes was granted from the Administration of Radioactive Substances Advisory Committee of the United Kingdom. Informed consent was obtained from all patients before entry into each of the studies.

Detailed methods for the three individual studies are described elsewhere (8, 13, 14). In brief, in a phase I dose-escalating study of CA4P, patients with abdominal metastases from a variety of primary tumors underwent dynamic PET scanning with ^{15}O-labeled water (8). PET scans were done immediately before and 30 min after administration of CA4P (n = 9). In the second study included in this analysis, paired perfusion scans were done before start of first cycle and a subsequent cycle of 5-fluorouracil and folinic acid chemotherapy (de Gramont regimen) in patients with colorectal cancer metastases. To evaluate the effects of N/C on perfusion and drug uptake (13), patients were treated with 60 mg/kg nicotinamide given orally 2 to 3 h before the second scan and inhaled carbogen (95% oxygen, 5% carbon dioxide) for 5 min before and then 5 min during the perfusion scan (n = 6). In the final cohort of patients, paired perfusion PET scans were done before and on day 8 after treatment with 5-fluorouracil and IFN-α to evaluate the pharmacodynamic effect of IFN on tissue and tumor perfusion (14). Patients with liver metastases from gastrointestinal malignancies were administered IFN-α s.c. (3 million units) on day 1, 4, 7, and 8 after the perfusion scan on day 1 (n = 6).

Scanning

All PET scans were done using an ECAT 931-08/12 PET (2D) scanner (CTI/Siemens) as described previously (8, 13, 14). Studies evaluating the effects of CA4P and the N/C on the vasculature were done using ^{15}O-labeled water ([^{15}O]H_2O) as the positron emitting tracer. After insertion of arterial and venous cannulae, a bolus of 600 MBq of [^{15}O]H_2O was injected i.v. and patients were scanned for 10 min. In the IFN study, dynamic tissue-perfusion scans were conducted with ^{15}O-labeled CO_2, which transfers to ^{15}O-labeled water instantaneously in the lungs (17) and was delivered via a facemask at an activity of 4 MBq/mL and a constant flow of 500 mL/min over 3.5 min. Continuous arterial sampling was carried out during scanning to measure blood radioactivity with discrete arterial samples taken for calibration. Dynamic measurements of the radioactivity were obtained from the arterial blood samples, tumor, normal liver, kidneys, and spleen. Blood flow and V_d (water) were calculated using established methods (18).

PET Data Analysis

Reconstructed PET images were analyzed using the image analysis software ANALYZE (19). Regions of interest were manually defined on tumor, kidney (focused on the renal cortex), liver, and spleen on summated PET images assisted by a recent computed tomography scan. Regions of interests defined on summated PET images were then applied to the dynamic PET data and mean voxel counts for the individual time-frames calculated. Mean frame voxel counts were plotted to obtain time-activity curves. The modified Kety-Schmidt equation (9, 20) was used to model plasma time-activity curves derived from continuous arterial blood sampling data during the scan (input) with tissue time-activity curve (output) to obtain perfusion variables, blood flow (mL $_{blood}$/min/mL $_{tissue}$), and V_d (water) (mL plasma/mL (cm^3) tissue or unitless). Variables for the liver are only an index of perfusion because the liver has a dual blood supply from the hepatic artery and portal vein, and are thus only approximated by the one compartment blood flow model (21).

Statistical Analysis

Descriptive statistics including mean, range of values, and SD were obtained. Groups were compared using Wilcoxon signed-rank test for matched pairs and correlations were analyzed using two-sided Pearson parametric testing. Significance levels are two tailed at 0.05 levels with 95% confidence intervals.

Results

Patients

Clinical and tumor characteristics of the 21 patients included in the analyses are summarized in Table 1. The median age was 58 years (range, 24–80 years). The majority of patients had colorectal cancer and the commonest tumor lesions analyzed were liver metastases. Pretreatment perfusion values from tumor and spleen were obtained from 20 patients and from 18 and 12 patients from renal cortex and liver, respectively. Data were unobtainable 30 minutes posttreatment in the CA4P study for 1 patient due to technical problems, and posttreatment tumor Vd (water) was not obtained for another patient due to measurement uncertainty. One patient in the N/C study only had normal tissue values due to the tumor size being too small (1.5 cm) for accurate assessment.

Published OnlineFirst February 10, 2009; DOI: 10.1158/1535-7163.MCT-08-1016

Table 1. Patients characteristics, type of cancer and tumor, and normal tissue analyzed

Study/patient	Age (y)	Gender	Primary site of disease	Lesions evaluated (no. of lesions)	Size of lesions (cm)*	Normal tissue evaluated
CA4P/1	46	F	Colorectal	Liver met. (1), Lung met. (1)	a = 4 b = 2	K, S
CA4P/2	27	F	Osteosarcoma	Liver met. (2), Lung met. (2)	a = 4 b = 4 c = 2 d = 2	K, S†
CA4P/3	44	F	Colorectal	Liver met. (1)	a = 5	K, S
CA4P/4	24	M	Hepatoma	Primary (1)	a = 10	K, S
CA4P/5	64	M	GIST	Liver met. (3)	a = 8 b = 6 c = 4	K, S
CA4P/6	47	M	Osophageal	Para-aortic nodes (1)	a = 4	K, S
CA4P/7	52	M	Renal cell	Primary (1)	a = 15	K
CA4P/8	59	F	Hepatoma	Primary (2)	a = 5 b = 5	K, S
CA4P/9	64	M	Colorectal	Liver met. (1)	a = 11	K, S
N/C/1	58	F	Colorectal	Liver met. (4)	a = 3 b = 3 c = 3 d = 2	K, S, L
N/C/2	74	M	Colorectal	Liver met. (2)	a = 4 b = 2	K, S, L
N/C/3	74	M	Colorectal	Liver met. (2)	a = 10 b = 2	K, S, L
N/C/4	47	F	Colorectal	Liver met. (2)	a = 6 b = 4	K, S, L
N/C/5	51	M	Colorectal	Liver met. (3)	a = 6 b = 3 c = 3	K, S, L
N/C/6	77	F	Colorectal	Lymph nodes (2)	a = 1.5, b = 1.5‡	K, S, L
IFN/1	67	M	Colorectal	Liver met. (2)	a = 3 b = 3	S, L
IFN/2	46	F	Colorectal	Liver met. (3)	a = 7 b = 5 c = 3	K, S, L
IFN/3	61	M	Gastric	Liver met. (1)	a = 3.5	K, S, L
IFN/4	54	F	Colorectal	Liver met. (2)	a = 10 b = 7	S, L
IFN/5	80	F	Colorectal	Liver met. (1)	a = 5	K, S, L
IFN/6	59	M	Colorectal	Liver met. (2)	a = 5 b = 6	S, L

Abbreviations: F, female; M, male; Liver met, liver metastasis; K, kidney; S, spleen; L, liver.
*a, b, c, and d refers to individual lesions analyzed.
†Patient scanned before intervention only.
‡Only liver metastases evaluated in the analysis of the effect of N/C.

Blood Flow and V_d (water) Before Intervention

Before any therapeutic intervention, mean tumor blood flow (0.41 mL $_{blood}$/min/mL $_{tissue}$) was significantly lower than renal (1.58 mL $_{blood}$/min/mL $_{tissue}$) and splenic blood flow (1.44 mL $_{blood}$/min/mL $_{tissue}$) and the hepatic perfusion index (0.57 mL $_{blood}$/min/mL $_{tissue}$). Mean V_d (water) was also significantly lower in tumors (0.69) compared with spleen (0.89; P = 0.001) and liver (0.94; P = 0.002) but not kidneys (0.77; P = 0.11). Mean regional blood flow and V_d (water) values with 95% confidence interval before any intervention are listed in Table 2. Correlation analysis revealed a significant relationship between blood flow and V_d (water) in tumor tissue (r = 0.62; P = 0.004). This association was, however, not seen in any of the normal tissues studied, in which minimal variations in V_d (water) were observed over a wide range of flow measurements as illustrated in Fig. 1.

Relationship between Changes in Blood Flow and V_d (water)

Table 3 summarizes mean blood flow and V_d (water) values before and after intervention in the different tissues examined. In tumor lesions, mean regional blood flow decreased significantly after treatment with CA4P (P = 0.039). A nonsignificant increase in tumor flow was observed after N/C (P = 0.19) and tumor flow remained largely unchanged after IFN (P = 1.0). In contrast, mean tumor V_d (water) did not change significantly after any intervention. Despite the association between blood flow and V_d (water) in tumors before intervention, no evidence of a relationship between the magnitude of absolute or relative changes (percentage change from baseline) in blood flow and V_d (water) due to treatment was observed (Table 4; Fig. 2).

In normal tissues, mean renal blood flow significantly reduced after CA4P (P = 0.031) and N/C (P = 0.031) and a

Table 2. Baseline regional blood flow and V_d (water) measurements from all studies combined

Tissue	No. of patients	Regional blood flow ($mL_{blood}/min/mL_{tissue}$)	V_d (water)
Tumor	n = 21	0.41* (0.28–0.54)	0.69* (0.57–0.81)
Kidney	n = 18	1.58† (1.30–1.85)	0.77† (0.71–0.82)
Spleen	n = 20	1.44 (1.03–1.96)	0.89 (0.84–0.96)
Liver	n = 12	0.57 (0.41–0.72)	0.94 (0.86–1.03)

NOTE: Data are mean (95% confidence interval).
*Mean value if more than one tumor lesion analyzed.
†Mean values from right and left kidney.

Published OnlineFirst February 10, 2009; DOI: 10.1158/1535-7163.MCT-08-1016

decrease in hepatic perfusion index was observed with N/C ($P = 0.031$). Together with a decrease in renal flow, a significant decrease in renal V_d (water) was also observed with CA4P ($P = 0.031$) and N/C ($P = 0.031$). In contrast to tumors, a significant correlation between changes in blood flow and V_d (water) was observed after treatment with N/C in kidneys and spleen and in spleen with CA4P (Table 4; Fig. 3).

Discussion

Tumor vessels are structurally and functionally abnormal. They do not follow the hierarchical branching pattern of normal vascular networks (22) and are disorganized with irregular diameters and lengths between branching points (3, 23). The vessel walls are poorly developed with an irregular and structurally abnormal basement membrane that has only loose association with endothelial cells and pericytes (24). The endothelial cells themselves are morphologically abnormal with variations in size and shape. They form an uneven luminal layer with wide junctions leading to high vascular permeability to macromolecules (25). As a result, the hydrostatic and oncotic pressures become almost equal between the intravascular and extra vascular space (26). Inadequate lymphatic drainage further contributes to interstitial hypertension, which increases toward the center in most solid tumors. As a consequence, tumor blood flow is low, more so in central tumor regions compared with tumor as a whole (27). Raised IFP is thought to be an important contributing factor in the generation of areas of low flow, inhibiting fluid exchange, and leading to vascular collapse. High IFP has also been shown to increase with increasing tumor size and has been identified as a main barrier to the delivery of therapeutic agents (26, 28, 29).

The PET measure, V_d (water) reflects the proportion of tissue volume that exchanges water with plasma at steady-state during a given period of observation (10, 30, 31). In normal tissues, the exchanging water space seems to be at least partially related to the fat content with a low V_d (water) in normal breast tissue (18) but higher values in the brain and abdominal organs such as kidney and spleen (8, 12, 18, 32–34). Low V_d (water) values in tumor lesions found in previous studies are thought to reflect areas of necrotic tissue, although no direct comparison with histologic tumor sections has been reported thus far. In a number of myocardial perfusion studies, the perfusable tissue index has been described as a variable for the identification of tissue viability (30, 31). Calculation of the perfusable tissue index is based on the measurement of V_d (water) from dynamic O-water data. Iida et al. (30) found a low perfusable tissue index in infarcted myocardium compared with normal regions. In a later study, the same group could correlate the distribution of tissue necrosis, identified by histochemical staining, with the perfusable tissue index in a canine model of myocardial infarction (35). We therefore interpret our finding of low V_d (water) in tumors reflective of both tissue necrosis and raised IFP.

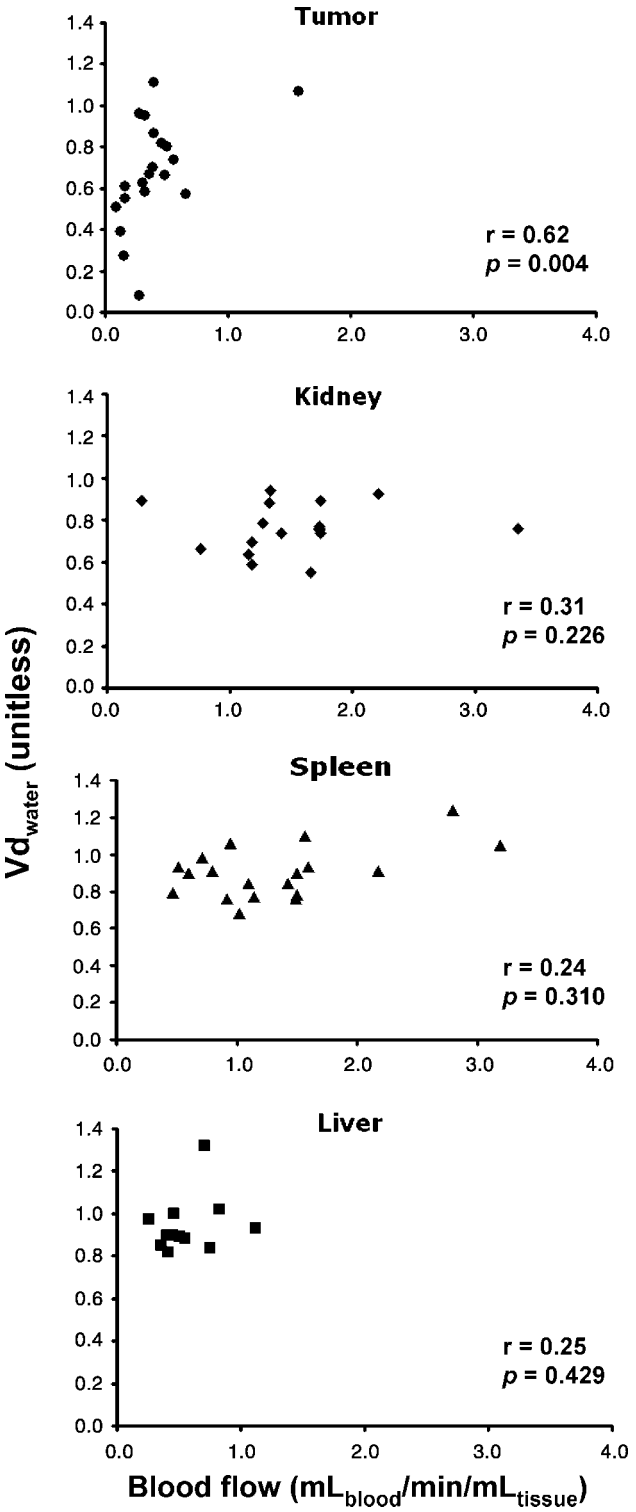

Figure 1. Relationship between blood flow and volume of distribution of water [V_d (water)] in tumor lesions and normal tissue before intervention. Mean values for the right and left kidney were taken in patients where both kidneys were analyzed. Due to the dual blood supply, blood flow in the liver is expressed as hepatic perfusion index. A significant correlation between these two variables is seen in tumors, whereas V_d (water) in normal tissue remains constant over a wide range of flow values.

Published OnlineFirst February 10, 2009; DOI: 10.1158/1535-7163.MCT-08-1016

Table 3. Mean blood flow and V_d (water) values before and after intervention in tumor, kidney, spleen, and liver

Tissue	Study	No. of patients	Mean blood flow ($mL_{blood}/min/mL_{tissue}$)			Mean V_d (water)		
			Pre	Post	*P*	Pre	Post	*P*
Tumor	CA4P	7	0.57	0.30	0.039*	0.78	0.75	0.437
	N/C	5	0.29	0.39	0.187	0.76	0.78	0.812
	IFN	6	0.33	0.31	1.000	0.48	0.56	0.156
Kidney	CA4P	8	1.44	1.20	0.031*	0.76	0.69	0.031*
	N/C	6	1.57	0.92	0.031*	0.81	0.64	0.031*
	IFN	3	2.14	1.56	0.250	0.68	0.74	0.750
Spleen	CA4P	7	1.02	0.68	0.078	0.88	0.84	0.109
	N/C	6	1.88	1.78	1.000	0.95	0.94	1.000
	IFN	6	1.72	1.19	0.125	0.90	0.90	0.844
Liver†	N/C	6	0.68	0.46	0.031*	0.99	0.90	0.218
	IFN	6	0.45	0.39	0.156	0.89	0.88	0.563

*Significant at the 0.05 level.
†Blood flow in the liver is expressed as hepatic perfusion index.

Accordingly, we interpret our findings of poor flow and limited exchangeable tissue in tumors to reflect impaired blood flow in a necrotic tumor microenvironment with a high IFP. The significant relationship between blood flow and V_d in tumors also underlies the importance and interdependence of these PET variables in tumor perfusion under these conditions.

In contrast to tumor tissue, the exchangeable volume of water in normal tissue was as expected higher than in tumors, with little variation in V_d (water) (Table 2). In normal tissues, blood flow is regulated by the autonomic nervous system, hormonal and local myogenic and metabolic factors that are responsible for optimal maintenance of tissue perfusion under physiologic conditions (homeostasis). As the capillary density in normal tissues is sufficient to meet the nutritional demand at any one time, the exchangeable tissue volume or V_d (water) is likely to be maximal and will largely depend on the tissue architecture. Therefore, when there is a need for additional nutritional requirement, there is little possibility for a further increase in the exchangeable volume of tissue. Consequently, homeostatic mechanisms result in an increase in capillary recruitment and/or an increase in flow per capillary leading to an increase in tumor blood flow per unit volume of tissue, as observed in the minimal change in V_d (water) over a range of tissue blood flow (Table 2) and the lack of correlation between flow and V_d (water) in normal tissues.

Having observed a significant relationship between the two variables in tumors before any therapy, we examined if changes in tumor blood flow were related to changes in V_d (water) after treatment with tumor vasculature targeting agents. Interestingly, we did not find that changes in tumor flow were associated with changes in V_d (water) in all the three studies assessed in this paper. We interpret this finding due to a variety of reasons including the mechanism of drug action and the presence of necrotic tumor tissue. With CA4P, a vascular disrupting agent known to cause a pronounced shutdown in blood flow to solid tumors (3, 9), an associated change in V_d (water) was not observed, despite a significant decrease in tumor blood flow. Although preclinical studies confirm a decrease in tumor blood flow with CA4P, the effect of CA4P on tumor IFP [and, hence, V_d (water)] are unclear. Tozer et al. (3) have suggested that decrease in tumor blood flow was due to increase in vascular permeability and IFP, whereas Horsmann et al. (36) felt that decrease in tumor flow was independent of increases in tumor IFP. In our analysis, lack of significant change in tumor V_d (water) despite a significant decrease in tumor blood flow with CA4P and noncorrelation of changes in flow with V_d (water) after CA4P was observed. These findings may thus be reflective of either a non–IFP-dependent change in tumor blood flow wherein there is a narrowing of tumor vasculature causing a decrease in flow but without changes in the exchanging

Table 4. Correlations between absolute and relative changes (percentage change form baseline) in blood flow and V_d (water) in different tissues according to individual studies

Tissue	Study	Lesions/pts*	Absolute changes		Relative changes	
			r	*P*	*r*	*P*
Tumor	CA4P	$n = 11$	−0.03	0.918	−0.02	0.959
	N/C	$n = 12$	0.05	0.867	0.37	0.237
	IFN	$n = 11$	0.15	0.663	−0.51	0.106
Kidney	CA4P	$n = 8$	−0.34	0.428	−0.37	0.359
	N/C	$n = 6$	0.89	0.033*	1.00	0.003†
Spleen	CA4P	$n = 7$	0.96	0.003*	0.86	0.024†
	N/C	$n = 6$	0.94	0.017*	0.83	0.057†
	IFN	$n = 6$	0.60	0.242	0.54	0.297
Liver	N/C	$n = 6$	−0.14	0.803	−0.08	0.019
	IFN	$n = 6$	0.43	0.419	−0.20	0.714

*In tumor tissue, individual lesions were correlated; in kidneys mean values for left and right kidney were used.
†Significant at the 0.05 level.

Published OnlineFirst February 10, 2009; DOI: 10.1158/1535-7163.MCT-08-1016

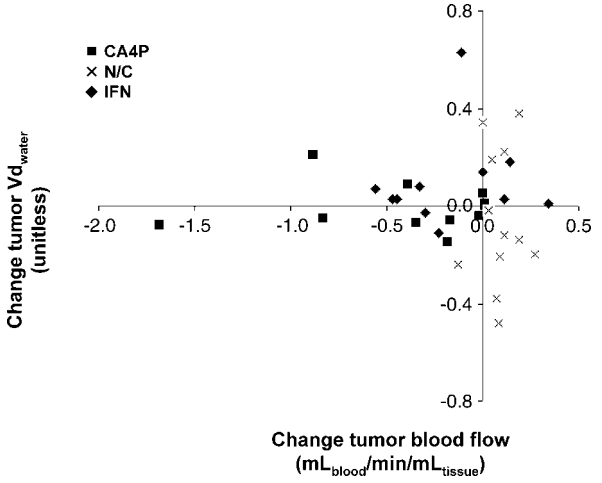

Figure 2. Changes in tumor blood flow are plotted against changes in tumor V_d (water). No evidence of a relationship in individual studies or all studies combined could be observed.

tumor volume with CA4P within the dose range evaluated in this study, or due to limited effect of CA4P on tumor IFP in a necrotic microenvironment. All tumors analyzed in this study were >2 cm and, therefore, contained significant necrosis and it was unlikely that CA4P would have any effect on tumor necrosis during the period evaluated. In patients who received N/C, although we observed some increase in tumor blood flow in four of five patients, no association between changes in flow and V_d (water) were seen and may similarly be indicative of drug action or a result of lack of influence on necrotic tumor areas. Our study group continues to evaluate changes in perfusion after vascular disrupting agents with molecular imaging and to understand the mechanistic effect of the vascular disrupting agents on tumor perfusion variables (37).

In contrast to tumors, where absolute decreases in V_d (water) were not observed together with decrease in flow after CA4P, we found that both renal flow and V_d (water) decreased after CA4P and also with N/C. However, decrease in renal flow was positively correlated with decrease in V_d (water) after N/C only but not with CA4P. These changes in renal perfusion in kidneys with N/C could reflect the mechanism of drug action due to the effect of hyperoxia on vascular diameter and associated changes in intracapillary hydrostatic pressure or due to vascular shunting in response to therapy. In this study, marked elevation of arterial blood pO2 also occurred (13), which has been shown to result in arteriolar vasoconstriction in normal tissue microvasculature, which may decrease both blood flow and exchangeable tissue and would explain our finding (38). In contrast to N/C, the differential results seen with CA4P on renal perfusion provides interesting insights into the mechanism of action of CA4P on renal vasculature. Despite a decrease in both flow and V_d (water) observed with CA4P in kidneys, the lack of a relationship between flow and V_d (water) changes in kidneys suggests that CA4P may causes changes in renal perfusion by two independent mechanisms. The effect of vascular disrupting agents on normal tissue perfusion is presently not well-understood, but the observation of cardiovascular changes observed in ongoing clinical trials requires further research in this area so that the drug therapeutic index is improved.

In conclusion, we have established an association between blood flow and fraction of perfused tissue, expressed as V_d (water), in tumors, but not normal tissue, indicating that in normal tissues V_d (water) is kept at a tissue optimum through intact autoregulation of the microvasculature. Furthermore, we have shown that in

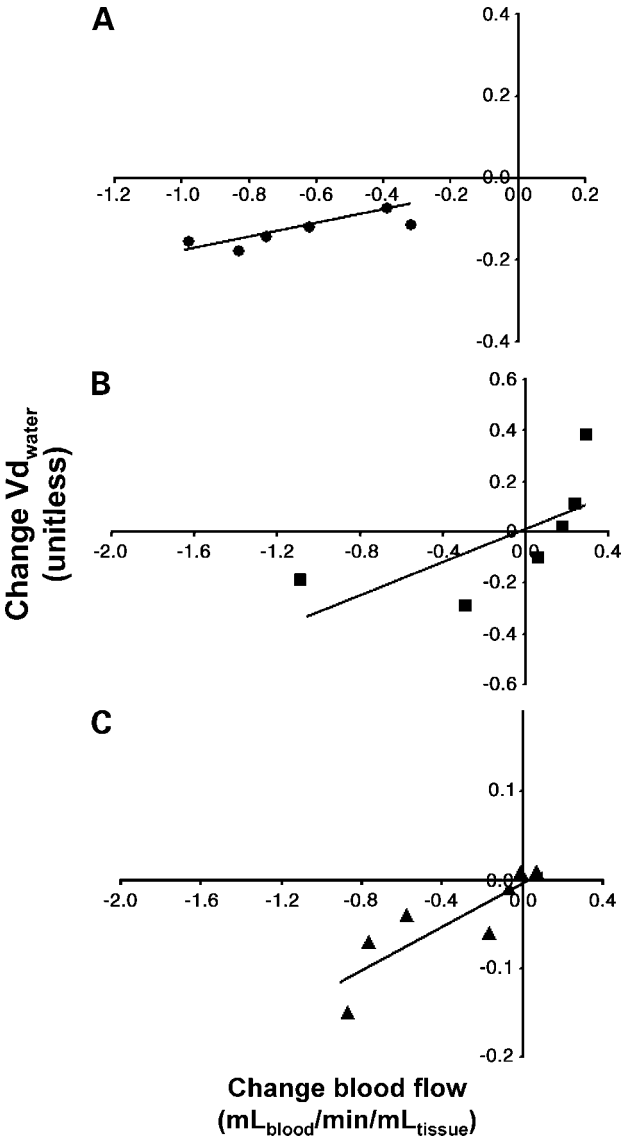

Figure 3. Changes in blood flow are plotted against changes in V_d (water) after administration of nicotinamide and carbogen in kidney (**A**), and in spleen (**B**), and after treatment with CA4P in spleen (**C**). In contrast to tumor tissue, changes in these two variables were related.

Published OnlineFirst February 10, 2009; DOI: 10.1158/1535-7163.MCT-08-1016

tumors, and contrary to normal tissues, changes in blood flow are not necessarily associated with changes in the perfused tissue fraction, a finding that we interpret to reflect presence of necrotic tissue, high interstitial pressure, or drug mechanism of action. Importantly, we have also highlighted the unique potential of dynamic PET perfusion imaging to measure tissue blood flow and fraction of perfused tissue separately. Evaluation of a combination of V_d (water) and flow provide valuable biomarkers for studying the mechanism of action of vascular targeted agents and will potentially aid anticancer drug development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Drs. RJ Harte, H. Anderson and N. Gupta whose data were used in this analysis.

References

1. Neri D, Bicknell R. Tumor vasculature targeting. Nat Rev Cancer 2005; 5:436 – 46.

2. Denekamp J, Hill S, Hobson B. Vascular occlusion and tumor cell death. Eur J Cancer 1983;19:271 – 5.

3. Tozer G, Kanthou C, Baguley B. Disrupting tumor blood vessels. Nat Rev Cancer 2005;5:423 – 35.

4. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer 2002;2:727 – 39.

5. Jain R. The next frontier of molecular medicine: delivery of therapeutics. Nat Med 1998;4:655 – 7.

6. Tozer G. Measuring tumor vascular response to antivascular and antiangiogenic drugs. Br J Radiol 2003;76:S23 – 35.

7. O'Connor J, Jackson A, Parker G, Jayson G. DCE-MRI biomarkers in the clinical evaluation of antiangiogenic and vascular disrupting agents. Br J Cancer 2007;96:189 – 95.

8. Anderson H, Yap J, Miller M, Robbins M, Jones T, Price P. Assessment of pharmacodynamic vascular response in a phase I trial of Combretastatin A4 Phosphate. J Clin Onc 2003;21:2823 – 30.

9. Anderson H, Price P. Clinical measurement of blood flow in tumors using positron emission tomography: a review. Nucl Med Commun 2002; 23:131 – 8.

10. Laking G, Price P. Positron emission tomography imaging of angiogenesis and vascular function. Br J Radiol 2003;76:S50 – 9.

11. Jain R. Delivery of novel therapeutic agents in tumors: physiologcial barriers and strategies. J Natl Cancer Inst 1989;81:570 – 6.

12. Anderson H, Yap J, Wells P, et al. Measurement of renal tumor and normal tissue perfusion using positron emission tomography in a phase II clinical trial of razoxane. Br J Cancer 2003;89:262 – 7.

13. Gupta N, Saleem A, Kötz B, et al. Carbogen and nicotinamide increase blood flow and 5-fluorouracil delivery but not 5-fluorouracil retention in colorectal cancer. Clin Cancer Res 2006;12:3115 – 23.

14. Harte R, Matthews J, O'Reilly S, et al. Tumor, normal tissue, and plasma pharmacokinetic studie of fluoruracil biomodulation with N-phosphonacetyl-L-aspartate, folinic acid, and interferon alfa. J Clin Oncol 1999;17:1580 – 9.

15. Dark G, Hill S, Prise V, Tozer G, Pettit G, Chaplin D. Combretastatin A-4, an agent that displays potent and selective toxicity towards tumor vasculature. Cancer Res 1997;57:1829 – 34.

16. Dickson PV, Hagedorn NL, Hamner JB, et al. IFN β-mediated vessel stabilization improves delivery and efficay of systemically administered topotecan in a murine neuroblastoma model. J Pediatr Surg 2007;42: 160 – 5.

17. West JB, Dollery CT. Uptake of oxygen-15-labeled CO2 compared with carbon-11-labeled CO2 in the lung. J Appl Physiol 1962; 17:9 – 13.

18. Wilson C, Lammertsma A, McKenzie C, Sikora K, Jones T. Measurements of blood flow and exchanging water space in breast tumors using positron emission tomography: a rapid and noninvasive dynamic method. Cancer Res 1992;52:1592 – 7.

19. Robb RA, Hanson DP. A software system for interactive and quantitative visualization of multidimensional biomedical images. Australas Phys Eng Sci Med 1991;14:9 – 30.

20. Cunningham V, Lammertsma A. Radioligand studies in brain: kinetic analysis of PET data. Med Chem Res 1994;4:79 – 96.

21. Ziegler S, Haberkorn U, Byrne H, et al. Measurement of liver blood flow using oxygen-15 labelled water and dynamic positron emission tomography: limitations of model description. Eur J Nucl Med 1996;23: 169 – 77.

22. Jain R. Molecular regulation of vessel maturation. Nat Med 2003;9: 685 – 93.

23. Konerding M, Malkusch W, Klapthor B, et al. Evidence for characteristic vascular patterns in solid tumors: quantitiative studies using corrosion casts. Br J Cancer 1999;80:724 – 32.

24. Baluk P, Morikawa S, Haskell A, Mancuso M, McDonald D. Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. Am J Pathol 2003;163:1801 – 15.

25. Hashizume H, Baluk P, Morikawa S, McLean JW. Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol 2000;156:1363 – 80.

26. Jain R. Intersitial transport in tumors: barriers and strategies for improvement. In: AACR 96th Annual Meeting: AACR Educational Book; 2005.

27. Rowell N, Flower M, Cronin B, McCready V. Quantitative single-photon emission tomography for tumor blood flow measurement in bronchial carcinoma. Eur J Nucl Med Mol Imaging 1993;7:591 – 9.

28. Huber PE, Bischof M, Jenne J, et al. Trimodal cancer treatment: beneficial effects of combined antiangiogenesis, radiation, and chemotherapy. Cancer Res 2005;65:3643 – 55.

29. Dickson PV, Hamner JB, Sims TL, et al. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. Clin Cancer Res 2007;13:3942 – 50.

30. Iida H, Rhodes CG, DeSilva R, et al. Myocardial tissue fraction - correction for partial volume effects and measure of tissue viability. J Nucl Med 1991;32:2169 – 75.

31. DeSilva R, Yamamoto Y, Rhodes CG, et al. Preoperative prediction of the outcome of coronary revascularization using positron emission tomography. Circulation 1992;86:1738 – 42.

32. Herscovitch P, Raichle M. What is the correct value for the brain-blood partition coefficient for water? J Cereb Blood Flow Metab 1985;5: 65 – 9.

33. Mankoff DA, Dunnwald LK, Gralow JR, et al. Blood flow and metabolism in locally advanced breast cancer: relationship to response to therapy. J Nucl Med 2002;43:500 – 9.

34. Saleem A, Yap J, Osman S, et al. Modulation of fluorouracil tissue pharmacokinetics by eniluracil: in-vivo imaging of drug action. Lancet 2000;355:2125 – 31.

35. Iida H, Tamura Y, Kitamura K, Bloomfield PM, Eberl S, Ono Y. Histochemical correlates of 15O-water-perfusable tissue fraction in experimental canine studies of old myocardial infarction. J Nucl Med 2000;41:1737 – 45.

36. Ley CD, Horsman MR, Kristjansen PE. Early effects of combretastatin-A4 disodium phosphate on tumor perfusion and interstitial fluid pressure. Neoplasia 2007;9:108 – 12.

37. Patterson D, Charnley N, Saleem A, et al. Phase I evaluation of vascular disrupting agent OXi4503. J Clin Oncol 2008;26:3551.

38. Duling BR. Microvascular response to alterations in oxygen tension. Circ Res 1972;31:481 – 9.

AACR American Association for Cancer Research

Molecular Cancer Therapeutics

Blood flow and V_d (water): both biomarkers required for interpreting the effects of vascular targeting agents on tumor and normal tissue

Barbara Kötz, Catharine West, Azeem Saleem, et al.

Mol Cancer Ther 2009;8:303-309. Published OnlineFirst February 10, 2009.