Carbogen and Nicotinamide Increase Blood Flow and 5-Fluorouracil Delivery but not 5-Fluorouracil Retention in Colorectal Cancer Metastases in Patients

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Abstract

Purpose: To examine whether carbogen and nicotinamide increases 5-fluorouracil (5-FU) delivery to colorectal cancer metastases.

Experimental Design: Six patients were scanned using positron emission tomography. Two scans were done to coincide with the start of separate chemotherapy cycles. At the second positron emission tomography session, 60 mg/kg nicotinamide was given orally 2 to 3 hours before 10-minute carbogen inhalation. In the middle of carbogen treatment, [^{15}O]H_2O (to measure regional tissue perfusion) and then [^{18}F]5-FU (to measure 5-FU tissue pharmacokinetics) were administered.

Results: Regions of interest were drawn in 12 liver metastases, 6 spleens, 6 livers, and 12 kidneys. Nicotinamide and carbogen administration increased mean blood pO_2 from 93 mm Hg (95% confidence interval, 79-198) to 278 mm Hg (95% confidence interval, 241-316; $P = 0.031$). Regional perfusion ($mL_{blood}/min/mL_{tissue}$) increased in metastases (mean change = 52%, range −32% to +261%, $P = 0.024$), but decreased in kidney (mean change = −42%, range −82% to −11%, $P = 0.0005$) and liver (mean change = −34%, range −43% to −26%, $P = 0.031$). 5-FU uptake at 3.75 minutes (m^2/mL) increased in tumor (mean change = 40%, range −39% to +196%, $P = 0.06$) and decreased in kidney (mean change = −25%, range −71% to 12%, $P = 0.043$). 5-FU delivery measured as K_1 increased in tumor (mean change = 74%, range −23% to +293%, $P = 0.0039$). No differences were seen in [^{18}F]5-FU tumor exposure (net area under curve) and retention.

Conclusion: Nicotinamide and carbogen administration can increase 5-FU delivery to colorectal cancer liver metastases. Despite an increase in perfusion and 5-FU delivery, the effects were not directly related and did not increase 5-FU retention or tissue exposure.

Although 5-fluorouracil (5-FU) dominates the chemotherapy of colorectal cancer, the response rate of advanced disease to the drug given as a single agent is only around 15% (1, 2). This poor response rate has stimulated research into approaches for increasing its therapeutic index, such as biochemical modulation (e.g., using folinic acid; ref. 1) and schedule alteration (e.g., continuous infusions rather than bolus administration, oral administration; ref. 2). The rationale behind changing the mode of 5-FU administration relates to its short biological half-life (10-20 minutes) and its S-phase-dependent activity, which limit the systemic exposure of drug and tumor cell kill (3, 4). Increasing the plasma levels of 5-FU can improve response and survival in patients (5, 6). However, additional improvements in 5-FU efficacy by increasing plasma drug levels are further limited by dose-limiting normal tissue toxicity, and other approaches for enhancing the efficacy of 5-FU are required.

It is postulated that the administration of nicotinamide and carbogen to patients with metastatic colorectal cancer should selectively increase the uptake of 5-FU into a tumor, by increasing tumor blood flow, without changing the systemic exposure to the drug. The nicotinamide and carbogen combination is being explored as an approach for reducing tumor hypoxia, an important cause of cancer treatment resistance (7), and improving radiotherapy response (8–11). Nicotinamide, an amide of vitamin B_3, can increase tumor blood flow via a mechanism thought to involve the stabilization of the tumor microvasculature (reducing contractility and fluctuations in blood flow; refs. 12, 13) and a decrease in

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Note: The protocol for this project was developed by Azeem Saleem, at the III workshop on "Methods in Clinical Cancer Research", jointly organized by federation of European Cancer Societies, American Association for Cancer Research and American Society of Clinical Oncology in Flims, Switzerland, 2001.

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interstitial fluid pressure (14). Additional mechanisms involving an effect of nicotinamide on tumor metabolism have also been implicated (9, 15). Carbogen (oxygen plus 3-5% carbon dioxide) increases blood flow and the amount of oxygen that can diffuse into tumors via a mechanism thought to involve the inhibition of hyperoxia-induced vasoconstriction (16–18). A combination of nicotinamide and carbogen was shown to increase the blood flow of human tumors (12, 19). For example, using laser Doppler probes, a 17% increase in human tumor blood flow was found 5 minutes after the start of carbogen inhalation, reaching a plateau at 8 to 10 minutes and rapidly dropping to baseline on cessation of carbogen inhalation (12). Moreover, in experimental tumors, carbogen increased the tumor uptake, half-life, and exposure of 5-FU, a finding attributed to an increase in the blood flow and a change in the pH gradient in the tumors (20).

The hypothesis behind the study reported here, therefore, was that nicotinamide and carbogen would increase 5-FU uptake in colorectal cancer liver metastases by increasing tumor blood flow and thus drug delivery. To test this hypothesis, the effect of nicotinamide and carbogen on tumor and normal tissue blood flow and 5-FU pharmacokinetics was studied using [^{15}O]H_2O and [^{18}F]5-FU positron emission tomography (PET). The measurement of tumor blood flow using [^{15}O]H_2O PET is an established technique (21, 22). The use of PET to study the *in vivo* pharmacokinetics of 5-FU in cancer patients has also been described (6, 23–25). Recently, we showed that [^{18}F]5-FU PET can provide a highly sensitive and quantitative pharmacokinetic analysis of 5-FU in tissues (24), and that the delivery of [^{18}F]5-FU to tumors is lower than to normal tissues, such as the spleen, liver, and kidney (26).

Materials and Methods

Patients and study outline. Ethical approval was obtained from the 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. Eight patients with colorectal cancer metastases receiving first-line chemotherapy with 5-FU/folinic acid were enrolled (five from the Hammersmith Hospital and three from the Charing Cross Hospital). Inclusion criteria included an ability to swallow and retain oral medication. Exclusion criteria included patients with a history of chronic pulmonary obstructive disease, which would impair their hypoxic respiratory drive and make them intolerant of the high oxygen content of carbogen. Chemotherapy consisted of 5-FU plus folinic acid given as the de Gramont regimen (folinic acid 200 mg/m^2 2-hour infusion, 5-FU 400 mg/m^2 bolus, and 600 mg/m^2 22-hour continuous infusion, days 1 and 2, repeated every 2 weeks) or modified de Gramont regimen (folinic acid 350 mg, 5-FU 400 mg/m^2 bolus and 2,800 mg/m^2 46-hour continuous infusion, repeated every 2 weeks). Patients underwent two PET scanning sessions, separated by a minimum of 2 weeks and a maximum of 2 months. Scanning was done in the early afternoon to avoid diurnal variation in the metabolism of 5-FU (27). The first scanning session occurred on day 1 of the de Gramont regimen and did not involve administration of nicotinamide and carbogen. [^{18}F]5-FU was given concurrently with the administration of therapeutic bolus 5-FU. The second scanning session occurred during a subsequent cycle of chemotherapy and patients were given 60 mg/kg nicotinamide orally 2 to 3 hours before scanning commenced. Carbogen (95% oxygen, 5% carbon dioxide) inhalation began 5 minutes before the start of both the [^{15}O]H_2O and [^{18}F]5-FU scans and continued for a further 5 minutes during each scan. Patients breathed carbogen (delivered at 10 L/min) through tightly fitting masks.

PET scanning. Imaging was done at the Hammersmith Hospital using an ECAT 931-08/12 PET (two-dimensional) scanner (CTI/Siemens, Knoxville, TN) allowing data collection from 15 planes with an axial field of view of 10.8 cm. Patients were positioned using data from a recent computed tomography scan and X-ray simulation, as described elsewhere (28). Positioning was confirmed using a short transmission scan after which a 20-minute transmission scan was carried out to correct for the attenuation of photons in the body. After insertion of arterial and venous cannulas, [^{15}O]H_2O (29) was injected i.v. and the patients were scanned for 10 minutes. Ten minutes after completion of the water scan, [^{18}F]5-FU (30) was administered as a 30-second i.v. bolus and the patients were scanned for a further 90 minutes.

Continuous arterial sampling was carried out during scanning to measure blood radioactivity (31). Discrete arterial samples were taken for calibration and to measure the contribution of parent and metabolite radioactivity in plasma and whole blood using reverse-phase high performance liquid chromatography (24). The arterial blood was also analyzed for CO_2 (mm Hg), O_2 (mm Hg), glucose (mmol/mL), and pH.

PET data analysis. Reconstructed PET images were analyzed and regions of interest (ROI) drawn using a recent computed tomography scan to assist delineation of tumor and normal tissue areas. ROIs were defined using ANALYZE image analysis software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) on summated images rather than individual time frames. The ROIs were defined on a minimum of three consecutive slices and the top and bottom planes were not sampled to reduce the effect of organ movement. The ROI was projected onto the dynamic scan to measure the radioactivity of individual time frames for each plane defined, and the mean voxel count for the midframe time was calculated. Voxel counts in kBq/mL were decay corrected and normalized for body surface area and injected activity, using the following equation:

$$\text{Corrected activity } (t)\ [\text{m2/mL}] = \frac{\text{Measured activity at time } (t)\ [\text{kBq/mL}] \times e\lambda t}{\text{Injected dose } [\text{kBq}]/\text{BSA}[\text{m2}]}$$

where λ is the decay constant (1.053×10^{-4} s^{-1} for ^{18}F and 5.6896×10^{-3} s^{-1} for ^{15}O) at time (t) and BSA is body surface area. The normalized data were plotted against midframe times to obtain a tissue activity curve. The area under the tissue activity curve ($AUC_{0\text{-}90\ \text{minutes}}$, m^2.min.mL^{-1}) was calculated as a measure of tissue exposure to the radiotracer. The standardized uptake value (SUV) was calculated at various time points to measure the uptake of the radiotracer into tissues. The SUV at 3.75 minutes ($SUV_{3.75}$) was used as a measure of early [^{18}F]5-FU uptake (32).

[^{18}F]5-FU PET data were also analyzed using kinetic modeling. Spectral analysis (33, 34) was used to obtain K_1, the clearance of [^{18}F]5-FU from plasma to tissue (mL_{blood}/min/mL_{tissue}) as a measure of 5-FU delivery. Spectral analysis was also used to obtain the impulse response function (IRF) at different time points from which the fractional retention of [^{18}F]5-FU (FRF) was obtained (FRF = $IRF_{60\ \text{minutes}}/IRF_{1\ \text{minute}}$) as a measure of 5-FU retention (with some contribution from radiolabeled catabolites of [^{18}F]5-FU in tissues).

The [^{15}O]H_2O PET data were modeled using the modified Kety-Schmidt equation, adjusted for use in dynamic [^{15}O]H_2O PET scans (21, 35), to measure regional perfusion (mL_{blood}/min/mL_{tissue}) and the volume of distribution (V_d) of water in normal tissue and tumor ROIs. Parameters for the liver are only an index of perfusion and V_d 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 (36). Cardiac output (L/min) was calculated from

the recorded on-line arterial blood $[^{15}O]H_2O$ curve, as described elsewhere (37).

Statistical analyses. Data were analyzed using the nonparametric paired Wilcoxon signed rank and Mann-Whitney tests for group comparison. Correlations were calculated using Pearson's correlation coefficient. All statistical tests were two sided and a 0.05 significance level was used.

Results

Patients. Eight patients were enrolled, four male and four female, with a mean age of 65 years (range 59-78 years). The mean injected activity of $[^{18}F]$5-FU was 345 MBq (range 136-403 MBq) and the mean injected dose of 5-FU was 380 mg/m^2 (range 300-400 mg/m^2). One of the eight patients did not tolerate carbogen, and another patient did not have a second scan due to disease progression. In the remaining six patients (three male, three female), no side effects were recorded after oral nicotinamide but two patients complained of minor difficulty with the tightness of the mask during carbogen inhalation. A total of 15 metastases in the six patients were scanned. Table 1 outlines the data obtained in six patients. Incomplete data were obtained on one liver metastasis that responded to treatment and became too small for measurement of flow and V_d and in two liver and two lymph node metastases of <2 cm size, which were subsequently found to be too small for spectral analysis.

Systemic physiology. Discrete arterial blood sampling during $[^{18}F]$5-FU scanning enabled analysis of a number of variables. The data obtained during the first and second scanning sessions were compared (Table 2). Figure 1 illustrates the change in blood pO_2 during carbogen inhalation after nicotinamide administration. Ten minutes after the start of carbogen inhalation, blood pO_2 increased from 93 mm Hg (range 77-117 mm Hg) to 278 mm Hg (range 213-319 mm Hg), and the increase was statistically significant ($P = 0.031$). There were no statistically significant changes in pCO_2, glucose, or pH. Analysis of $[^{15}O]H_2O$ PET data showed a nonsignificant 7% reduction in cardiac output.

Tissue perfusion. Figure 2 illustrates an abdominal computed tomography showing an axial slice and corresponding baseline $[^{15}O]H_2O$ and $[^{18}F]$5-FU scans for one of the patients enrolled in the study. Analysis of the $[^{15}O]H_2O$ images yielded measurements of perfusion and the V_d of water for ROIs drawn in tumor and normal tissues before and after administration of nicotinamide plus carbogen (Table 3). As expected, perfusion in the liver metastases was lower than in normal liver parenchyma. In the two lymph node metastases, perfusion was lower than in normal kidney or spleen, but higher than the hepatic perfusion index or perfusion in the liver metastases. Nicotinamide and carbogen administration led to an increase in perfusion in 10 of 12 liver metastases (Fig. 3) with a mean increase of 52% (range −32% to +261%, $P = 0.024$; $n = 12$). In contrast, there was a considerable decrease in perfusion of the two lymph node metastases with a mean change of −60%.

In normal tissue, the hepatic perfusion index decreased with a mean change of −34% (range −43% to −26%; $P = 0.031$). Changes in the spleen were variable with no noteworthy change in mean splenic perfusion (mean 3%, range −34% to +26%, $P = 1$). There was a decrease in renal perfusion (12 kidney ROIs; right and left kidney taken) with a mean change

Table 1. Scan data obtained for six patients with 15 metastases

	Metastases	Size (cm)	$[^{15}O]H_2O$, pre-C/N	$[^{15}O]H_2O$, post-C/N	$[^{18}F]$5-FU, pre-C/N	$[^{18}F]$5-FU, post-C/N
Patient 1						
Met a	Liver	3	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met b	Liver	3	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met c	Liver	3	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met d	Liver	2	Flow, V_d	Flow, V_d		
Patient 2						
Met a	Liver	4	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met b	Liver	2	Flow, V_d		AUC, $SUV_{3.75}$	AUC, $SUV_{3.75}$
Patient 3						
Met a	Liver	>10	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met b	Liver	2	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$
Patient 4						
Met a	Liver	6	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met b	Liver	4	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Patient 5						
Met a	Liver	>6	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met b	Liver	3	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Met c	Liver	3	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$, K_1, FRF	AUC, $SUV_{3.75}$, K_1, FRF
Patient 6						
Met a	Lymph node	1.5	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$	AUC, $SUV_{3.75}$
Met b	Lymph node	1.5	Flow, V_d	Flow, V_d	AUC, $SUV_{3.75}$	AUC, $SUV_{3.75}$

Abbreviations: C/N, carbogen and nicotinamide; Met, metastasis; V_d, volume of distribution of water; AUC, area under the tissue activity curve; SUV, standardized uptake value; K_1, clearance from plasma to tissue; FRF, fractional retention of 5-fluoruracil.

Table 2. Physiologic variables

Parameter	Scan 1	Scan 2	P*
pO_2 (mm Hg)	93.3 (79-108)	278.2 (241-316)	0.031
pCO_2 (mm Hg)	35.9 (33.3-38.4)	36.3 (30.6-42.4)	0.56
Glucose (mmol/L)	6.2 (4.3-8.0)	6.2 (3.8-8.6)	0.86
pH	7.45 (7.42-7.47)	7.45 (7.40-7.50)	1.00
Cardiac output (L/min)	3.42 (2.93-3.90)	3.11 (2.35-3.88)	0.43

NOTE: During the second scan, patients received nicotinamide and carbogen. Values are expressed as mean (95% confidence interval). Data for pO_2, CO_2, glucose, and pH are for the 5-minute time point (i.e., 10 minutes after the start of carbogen inhalation), the time at which the pO_2 values during the second scan were highest.
*Wilcoxon signed rank test of the data obtained in the first and second scans.

of −42% (range −82% to −11%; $P = 0.0005$). The V_d of water was also lower in the liver metastases than in normal tissue. Despite the increase in perfusion, no significant change in the V_d of water was seen in the liver metastases (mean change −1%, range −48% to +66%, $P = 0.52$; $n = 12$), and a mean decrease of 32% was seen in the lymph node metastases. A decrease in the V_d of water observed in kidney (mean change of −17%; range −44% to 2%; $P = 0.001$) was not seen in spleen or liver (Fig. 3).

Effect of nicotinamide and carbogen on 5-FU pharmacokinetics. Administration of nicotinamide and carbogen increased the early uptake of [^{18}F]5-FU to the colorectal liver metastases, seen as an increase in tumor $SUV_{3.75}$ (mean change 40%, range −39% to 196%, $P = 0.064$). There were no significant changes in either spleen or liver $SUV_{3.75}$, but a significant decrease was seen in kidney (mean change −25%, range −71% to 12%, $P = 0.043$; Table 4). Figure 4 outlines the changes in $SUV_{3.75}$ in the 12 liver metastases and normal tissue. No statistically

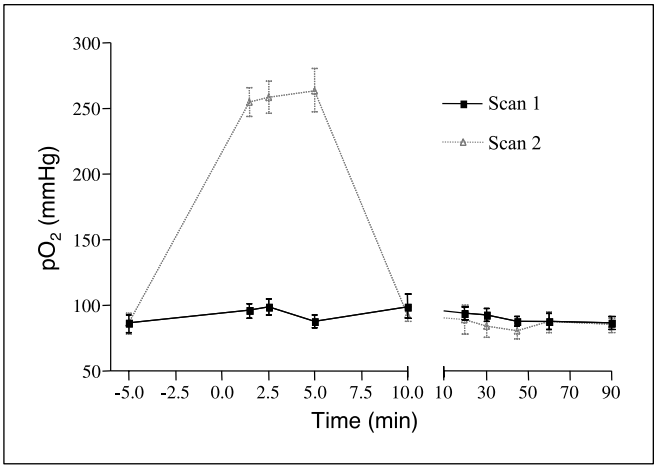

Fig. 1. Effect of nicotinamide and carbogen on arterial blood partial pressure of oxygen (pO_2). Points, mean from six patients; bars, SE. During the second scan, patients received nicotinamide and carbogen. Measurements were made over 95 minutes and for the second scan started 5 minutes before the start of carbogen inhalation.

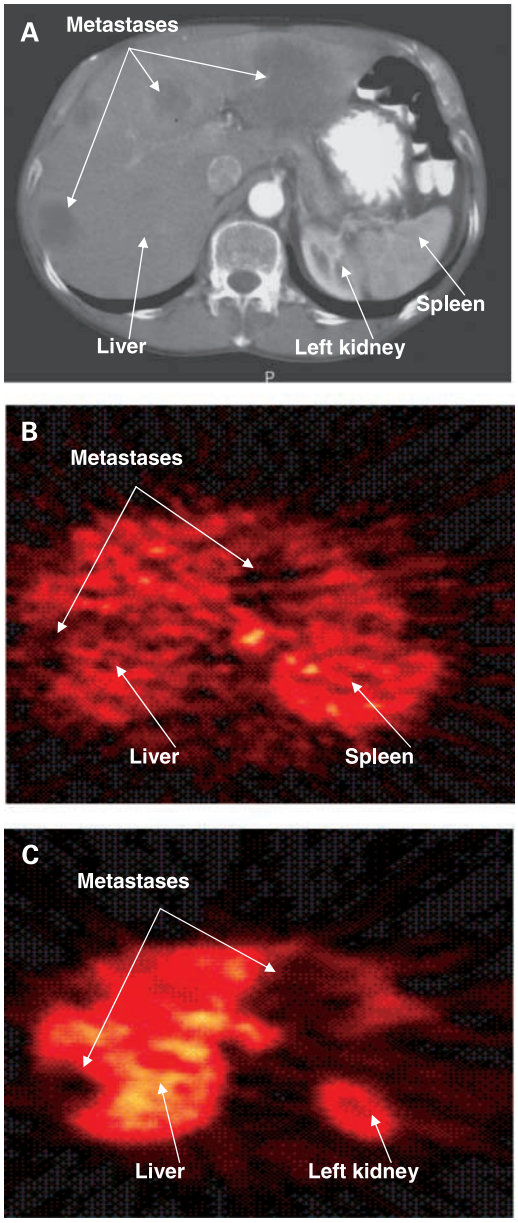

Fig. 2. Typical transabdominal computed tomography (*A*), [^{15}O]H_2O (*B*), and [^{18}F]5-FU (*C*) images for a patient with metastatic colorectal cancer. Arrows, analyzed tissues.

significant effects of nicotinamide and carbogen were seen on 5-FU exposure measured as $AUC_{0\text{-}90\ minutes}$.

The effect of nicotinamide and carbogen on the delivery and retention of 5-FU was also measured using variables derived by kinetic modeling (K_1 and FRF, respectively), which use plasma input functions corrected for labeled metabolites (Table 5). Parameters were obtained for 10 liver metastases, but not for kidney or liver due to a poor fit to the data. Figure 5 illustrates the effect of nicotinamide and carbogen on 5-FU delivery measured as K_1. There was a statistically significant increase in K_1 with a mean change of 74% (range −23% to 293%, $P = 0.0039$). Using the kinetic

Table 3. Regional perfusion and water V_d measurements

Tissue	Regional perfusion ($mL_{blood}/min/mL_{tissue}$)			V_d (mL_{blood}/mL_{tissue})		
	Scan 1	Scan 2	P*	Scan 1	Scan 2	P*
Liver metastasis (n = 12)	0.27 (0.08-0.56)	0.36 (0.13-0.67)	0.024	0.78 (0.53-1.09)	0.73 (0.41-1.02)	0.52
LN metastasis (n = 2)	0.87 (0.85-0.88)	0.35 (0.35-0.35)	N/A	1.04 (1.01 - 1.06)	0.70 (0.70-0.71)	N/A
Spleen (n = 6)	1.88 (0.82-2.94)	1.78 (1.06-2.50)	1.00	0.94 (0.75-1.14)	0.94 (0.83-1.04)	1.00
Kidney (n = 12)	1.57 (1.31-1.82)	0.92 (0.67-1.27)	0.0005	0.81 (0.73-0.90)	0.68 (0.58-0.79)	0.001
Liver (n = 6)	0.68 (0.40-0.96)	0.46 (0.24-0.67)	0.031	0.99 (0.81-1.81)	0.90 (0.80-0.99)	0.22

NOTE: During the second scan, patients received nicotinamide and carbogen. Values are expressed as mean (95% confidence interval).
Abbreviations: LN, lymph node; n, number; N/A, not applicable.
*Wilcoxon signed rank test of the data obtained in the first and second scans.

modeling-derived variable, the retention of 5-FU was around 10-fold higher in tumor compared with spleen and the difference was statistically significant. Nicotinamide and carbogen administration did not affect the retention of 5-FU measured as FRF in tumor and spleen.

Relationship between perfusion and 5-FU pharmacokinetics. The relationships between regional perfusion and 5-FU pharmacokinetics were studied. Before intervention, a significant correlation was found between perfusion in the 12 liver metastases and $SUV_{3.75}$ (r = 0.69, P = 0.013, n = 12), but only

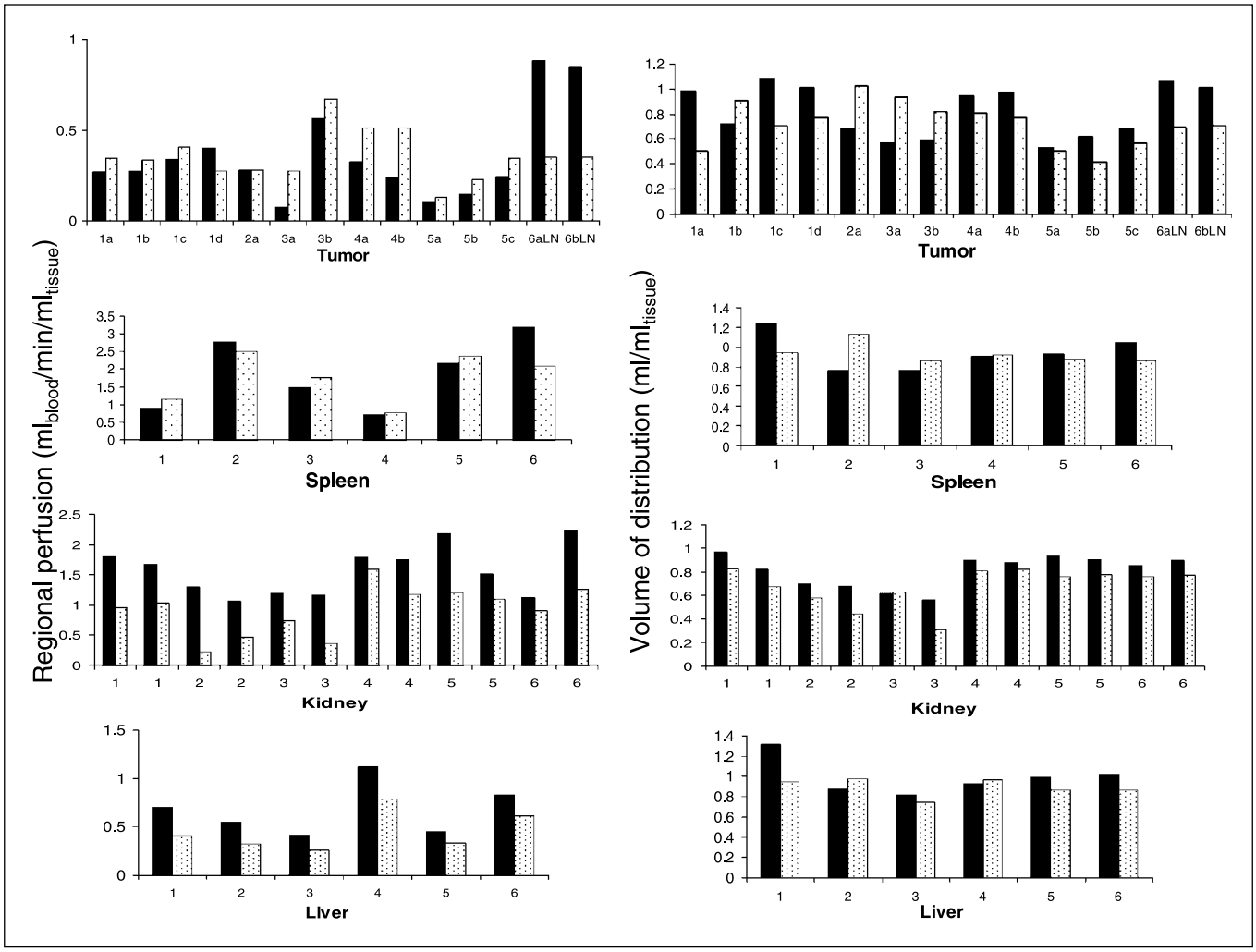

Fig. 3. Effect of nicotinamide and carbogen on regional perfusion (*left column*) and volume of distribution of water (V_d; *right column*) in tumor, spleen, kidney (*right* and *left*), and liver. Data for 12 liver and 2 lymph node (*LN*) metastases analyzed in six patients (*1* to *6,* individual patients; *a* to *d,* separate metastases analyzed). Black columns, without nicotinamide and carbogen; dotted columns, with nicotinamide and carbogen. Measurements were not obtained for all metastases (Table 1).

Table 4. 5-FU uptake (SUV) and tissue exposure ($AUC_{0\text{-}90\ minutes}$)

Region	*n*	$SUV_{3.75} \times 10^{-5}$			$AUC_{0\text{-}90\ minutes}$		
		Scan 1	Scan 2	*P**	Scan 1	Scan 2	*P**
Liver metastasis	12	7.01 (3.5-9.0)	8.32 (5.7-10.9)	0.064	0.45 (0.28-0.60)	0.41 (0.25-0.56)	0.32
Spleen	6	12.32 (10.2-14.5)	12.52 (10.1-15.0)	0.84	0.25 (0.21-0.29)	0.27 (0.17-0.37)	0.84
Kidney	12	27.0 (18.0-36.0)	17.7 (15.0-21.0)	0.043	1.29 (0.86-1.70)	1.21 (0.89-1.50)	0.67
Liver	6	15.89 (13.0-18.0)	14.73 (13.0-17.0)	0.44	1.62 (1.30-1.90)	1.62 (1.00-2.20)	0.56

NOTE: The units for SUV are m^2/mL and for $AUC_{0\text{-}90\ minutes}$ m^2.min.mL^{-1}. During the second scan, patients received nicotinamide and carbogen. Values are expressed as mean (95% confidence interval).
*Wilcoxon signed rank test of the data obtained in the first and second scans.

a nonsignificant trend between V_d of water and $SUV_{3.75}$ ($r = 0.53$, $P = 0.073$, $n = 12$; Fig. 6). As expected, a significant correlation was also seen between tumor $AUC_{0\text{-}90\ minutes}$ and regional perfusion ($r = 0.83$, $P = 0.0002$, $n = 14$) and water V_d ($r = 0.76$, $P = 0.002$, $n = 14$). No statistical significant correlation with K_1 and FRF could be seen prior or following therapy and no significant correlations between regional perfusion and uptake or tissue exposure to 5-FU could be seen following intervention. Despite increases in mean $SUV_{3.75}$ and K_1, no correlations were seen between changes in tumor regional perfusion or V_d of water and 5-FU delivery, exposure, or retention (Table 6).

Discussion

Many studies have investigated methods aiming to improve the efficacy of 5-FU in advanced colorectal cancer. The approaches studied include biomodulation, schedule alterations, and regional infusion techniques. A nonclinical study showed, using magnetic resonance spectroscopy (MRS), that carbogen breathing increased the uptake, retention, and cytotoxicity of 5-FU in hypoxic murine RIF-1 tumors (20). Two independent mechanisms were proposed for the increases: alterations in tumor blood flow and tumor pH gradient leading to improved 5-FU distribution and carbogen-induced enhanced formation of 5-FU anabolites (20). The purpose of the study reported here was to determine whether similar beneficial effects would occur in man following the administration of nicotinamide and carbogen to patients with metastatic colorectal cancer. The advantages of PET over MRS are its superior sensitivity and better spatial and temporal resolution. Although MRS can distinguish parent compounds from radiolabeled metabolites, arterial blood sampling and the application of kinetic modeling analysis with PET allows quantification of relevant pharmacokinetic variables.

As expected, arterial pO_2 rose rapidly in response to carbogen administration and returned to normal within a few minutes after discontinuation of the inhalation. This finding agrees with data from a nonclinical study where arterial pO_2 returned to pretreatment levels 10 minutes after discontinuation of hyperoxia (38). In addition, in both studies, the blood pH remained unchanged. However, in the animal model, there was a 36% increase in arterial pCO_2 in response to carbogen, which remained elevated for at least 10 minutes after the cessation of carbogen inhalation. As there was a lack of change in arterial pCO_2 in our study, it remains unclear whether the observed changes in blood flow are indeed due to the significant temporary increase of arterial pO_2 and question the contribution of the inspired CO_2 fraction. However, further work is needed to clarify the complex physiologic changes that might occur in response to nicotinamide and carbogen administration in man.

Using PET, we were able to investigate concurrently a number of normal tissues, namely kidney, liver, and spleen. Tissue type–dependent changes in perfusion were seen, consistent with a study in an experimental tumor model, where perfusion of the liver and kidney significantly decreased and an increase in

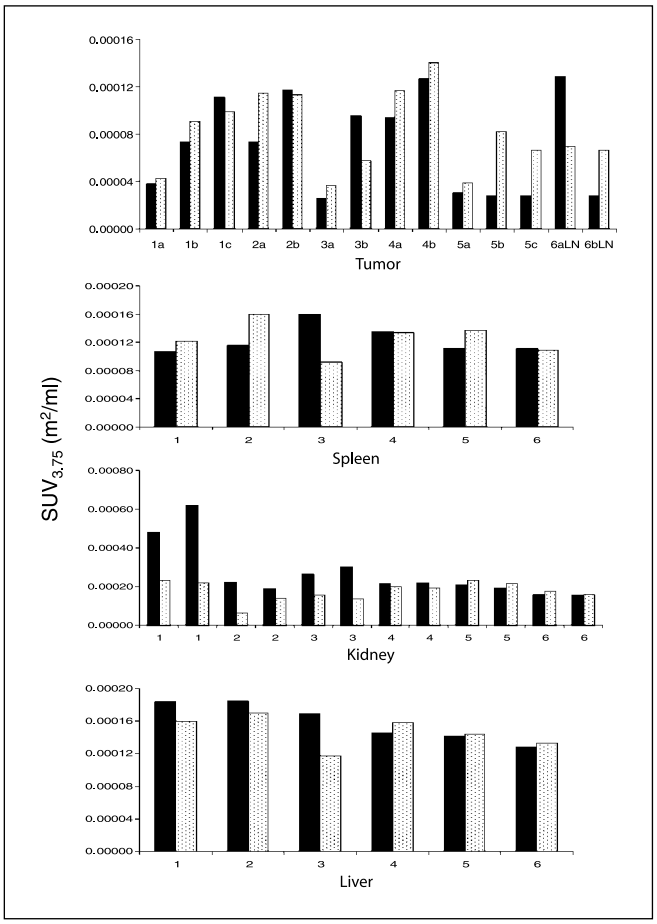

Fig. 4. Effect of nicotinamide and carbogen on 5-FU uptake (measured as $SUV_{3.75}$) in different tissues. Data for 14 metastases in six patients. Black columns, without nicotinamide and carbogen; dotted columns, with nicotinamide and carbogen. Measurements were not obtained for all metastases (see Table 1).

Table 5. 5-FU delivery and retention calculated using kinetic modeling

Tissue	*n*	K_1 ($mL_{blood}/min/mL_{tissue}$)			FRF		
		Scan 1	Scan 2	*P**	Scan 1	Scan 2	*P**
Tumor	10	0.089 (0.057-0.12)	0.136 (0.078-0.18)	0.0039	0.35 (0.19-0.49)	0.32 (0.17-0.47)	0.18
Spleen	6	0.71 (0.52-0.89)	0.62 (0.44-0.80)	0.44	0.031 (0.017-0.047)	0.028 (0.013-0.043)	0.84

NOTE: During the second scan, patients received nicotinamide and carbogen. Values are expressed as mean (95% confidence interval).
*Wilcoxon signed rank test of the data obtained in the first and second scans.

perfusion in the spleen was noted after carbogen inhalation (17). In the animal study, the increase in splenic perfusion was further attenuated by the addition of nicotinamide and largest in treatment with nicotinamide only. We saw a dramatic decrease in renal flow in response to nicotinamide and carbogen. This reduction in kidney perfusion, and corresponding decrease in water V_d, suggests that in man renal blood vessels shut down in response to nicotinamide and carbogen. A reduction of flow index was also seen in the liver where there was also a tendency for V_d to decrease (Fig. 3). As cardiac output was maintained, the effect on kidney and liver perfusion indicate mechanisms of autoregulation in these organs. Acute local blood flow control is normally achieved by rapid changes in local constriction of arterioles to maintain a constant supply of oxygen to the tissue (39). As there was no significant increase of pCO_2 in this study, the changes seen in normal tissue are likely to stem from the local effects of hyperoxia. This suggestion is further supported by the lack of changes in liver or kidney perfusion with nicotinamide only in the animal model. In analogy with the animal study, the lack of change in blood flow in the spleen could be explained by suspension of any improvement in flow with nicotinamide by the vasoconstricting effect of the increased pO_2; however, evaluation of the exact mechanism would have required separate studies with either nicotinamide or carbogen inhalation.

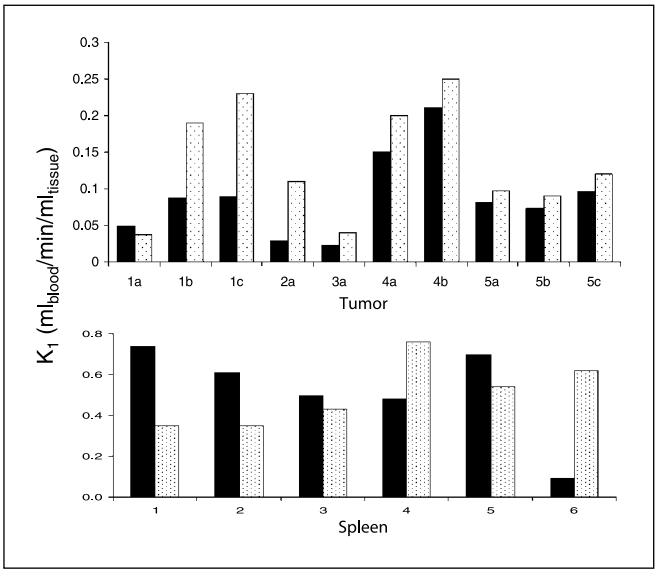

Fig. 5. Effect of nicotinamide and carbogen on 5-FU delivery (measured as K_1) in tumor and spleen. Data for 10 metastases in six patients. Black columns, without nicotinamide and carbogen; dotted columns, with nicotinamide and carbogen. Measurements were not obtained for all metastases (see Table 1).

Our study confirms the inferior blood flow in colorectal liver metastases compared with normal tissues, such as spleen, kidney, and liver. This finding, together with the significant correlation between tumor perfusion and tissue exposure of 5-FU before carbogen and nicotinamide administration, supports the theory that impaired tumor perfusion contributes to the poor response of colorectal cancer metastases to chemotherapy (6). As noted in previous studies, the effects of nicotinamide and carbogen on tumor perfusion seem to be site dependent (40). We saw a 32% increase in perfusion of the liver metastases in response to nicotinamide and carbogen administration, whereas perfusion of the two lymph node metastases decreased. A 22% increase was measured by laser Doppler probes in skin metastases and s.c. nodes in advanced human tumors (12), but no changes in perfusion were found in human glioblastoma or normal brain when evaluated with single photon emission computed tomography (41).

The changes in perfusion observed in normal tissues and tumor in this study suggests an augmentation of blood flow in tissues less capable of local vascular control mechanisms, such as tumor. This finding is consistent with the theory that the steal, or in this case better described as "gain" phenomenon, is the dominant mechanism for redistribution of host blood flow to tumor (42). The V_d of water in tumors did not alter in response to nicotinamide and carbogen administration, suggesting that there was no recruitment of previously nonfunctioning vessels in the liver metastases. In animal models, vasoconstrictor-induced increase in the mean blood pressure has led to increases in tumor blood flow but also interstitial fluid pressure (42). High interstitial fluid pressure may also explain the lack of correlation between tumor blood flow and 5-FU delivery measured as K_1 at baseline. However, the tumor is likely to be responding in a complex and heterogeneous manner. Parts of the tumor might be responding by an increase in flow with an increase in 5-FU extraction, whereas others might respond with an increase in V_d but no changes in flow. For example, in one tumor, there was a large increase in K_1, no change in flow, but an increase in V_d and, therefore, more of tumor tissue being perfused. In another patient, there was a large increase in flow and V_d, but only a modest increase in K_1, which is likely to be due a reduction in the single-pass extraction fraction due to the very high flow (43). The available data suggest that in humans, perfusion of liver metastases increases in response to nicotinamide and carbogen, with the underlying mechanisms requiring further research.

The early uptake of [^{18}F]5-FU (measured as $SUV_{3.75}$) was higher in the normal tissues studied than in the liver metastases, a finding that mirrored the higher normal tissue perfusion. This observation, together with a significant correlation between tumor perfusion and $SUV_{3.75}$ before

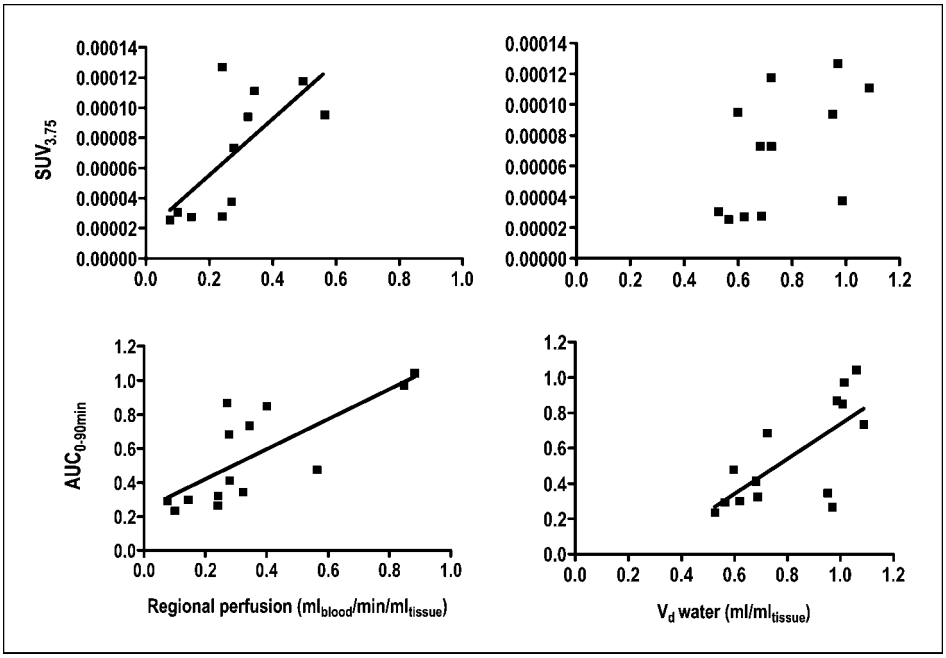

Fig. 6. Correlations between $SUV_{3.75}$ and perfusion (*top left*), AUC and perfusion (*bottom left*), $SUV_{3.75}$ and V_d water (*top right*), and AUC and V_d water (*bottom right*) in 12 liver and 2 lymph node metastases before intervention.

intervention, suggests that [^{18}F]5-FU uptake is related to tissue perfusion in liver metastases. The data show that nicotinamide and carbogen can increase tumor perfusion, although the effect on [^{18}F]5-FU uptake and delivery varied between individual metastases. These findings are similar to changes observed in mice, where only large tumors showed increased uptake of 5-FU in response to carbogen (20). In our study, all five liver metastases larger than 4 cm had increased K_1 and $SUV_{3.75}$ values. In contrast, the effect of carbogen on 5-FU uptake and delivery in tumors <4 cm in size was variable and was not consistent with changes in regional tumor perfusion.

Table 6. Changes in flow, $SUV_{3.75}$, and K_1 in metastases

	Metastases	Flow	$SUV_{3.75}$	K_1
Patient 1				
Met a	Liver	29	13	−23
Met b	Liver	21	24	118
Met c	Liver	18	−11	158
Met d	Liver	−32		
Patient 2				
Met a	Liver	0	57	293
Met b	Liver		−4	
Patient 3				
Met a	Liver	261	43	74
Met b	Liver	19	−40	
Patient 4				
Met a	Liver	59	24	33
Met b	Liver	113	10	19
Patient 5				
Met a	Liver	29	26	18
Met b	Liver	58	196	23
Met c	Liver	44	138	25
Patient 6				
Met a	Lymph node	−60	−46	
Met b	Lymph node	−59	137	

NOTE: All values are percentage change from baseline scan without carbogen and nicotinamide.
Abbreviation: Met, metastasis.

The tissue exposure and retention of [^{18}F]5-FU (measured as $AUC_{0\text{-}90\ minutes}$ or FRF, respectively) was unaffected by nicotinamide and carbogen, even in the larger tumors studied. This finding contrasts with that of McSheehy et al. (20) who showed a carbogen-induced increase in 5-FU retention measured using MRS in murine RIF-1 tumors. The group related the increase in 5-FU retention to a differential increase in extracellular versus intracellular pH, thus creating a proton pump gradient favoring intracellular retention of 5-FU in tumors (44). The lack of change in intratumoral retention of [^{18}F]5-FU may be due to the 20-minute shorter duration of carbogen breathing in our study, the lack of change in blood pH, or a cancellation of the increased delivery because of an increased rate of washout. Indeed, we have not evaluated the transport out of the intracellular volume of the metastases (k_{out}) and it has previously been shown that trapping of 5-FU with an increase in AUC can only be expected if the transport into the metastases (k_{in}) outweighs k_{out} (23). In our study, a significant correlation between K_1 and $AUC_{0\text{-}90\ minutes}$ before, but not after, nicotinamide and carbogen (data not shown) suggests that the increased flow may also lead to an increased washout. However, further study is required to examine the effects of nicotinamide and carbogen on 5-FU extraction from tissues. Future studies with MRS would also be of interest to identify any chemical species for which there might be an increased exposure or retention.

An important criterion to be considered is the potential effect of nicotinamide and carbogen on normal tissue toxicity. A recent paper highlighted an unexpected MRS detection of 5-FU anabolites in the metastasis-free liver of a patient, raising the issue of increased hepatocellular toxicity with concurrent

carbogen breathing (45). However, studies in rats did not find any carbogen-induced change in the liver or small intestine levels of 5-fluoro-2′-deoxyuridine-5′-monophosphate measured using MRS. Our own study has corroborated the rat studies and indicates a lack of increased delivery and retention of [^{18}F]5-FU in kidney, spleen and liver.

In summary, this study provides evidence that manipulation of tumor perfusion by nicotinamide and carbogen can increase [^{18}F]5-FU delivery in liver metastases. A significant reduction in renal blood flow and hepatic perfusion index in response to nicotinamide and carbogen suggests an indirect effect on colorectal cancer metastases in the liver, possibly via redistribution of blood from vascular normal liver parenchyma to tumor. The methodologies developed here should be useful for the assessment of other approaches being developed to improve the delivery of chemotherapeutic drugs to human tumors, and for the appraisal of the effects of nicotinamide and carbogen on other compounds such as molecularly targeted agents. The lack of effect of nicotinamide and carbogen on 5-FU exposure and retention in tumors and the dramatic effect on renal perfusion suggest further studies are required.

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