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Original Paper

Effect of Regional Angiotensin II Infusion on the Relationship Between Tumour Blood Flow and Fluorouracil Uptake in a Liver Metastasis Animal Model

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The aim of this study was to assess the relationship between tumour:liver blood flow and 5-fluorouracil (5-FU) uptake ratios in a hypovascular liver metastasis animal model, and examine whether they were similarly affected by a 5 min infusion of angiotension II via the hepatic artery. Tumour:liver blood flow ratio was measured using the isotope tracer ^{64}Copper (II)-pyruvaldehyde bis(n-4 methyl thiosemicarbazone, and 5-FU was tritiated. There was a wide variation in tumour:liver blood flow and 5-FU uptake ratios which could only partly be explained by between animal variation, and was not related either to individual tumour size or overall tumour burden within the liver. There was a close correlation ($r = 0.957$, $P < 0.0001$) between tumour:liver blood flow and 5-FU uptake ratios. Angiotensin II infusion significantly increased tumour:liver blood flow (nested analysis of variance, $P = 0.05$) but not 5-FU uptake ($P = 0.29$) ratios. There was a poor correlation ($r = 0.51$, $P = 0.13$) between tumour:liver blood flow and 5-FU uptake ratios with angiotensin II infusion. Thus, despite an increased 5-FU blood concentration arising from angiotensin-induced reduction in blood flow at constant 5-FU infusion dose, tumour:liver 5-FU uptake ratio did not increase as expected, and there ceased to be a significant correlation between tumour:liver blood flow and 5-FU uptake ratios. We conclude that the vasoactive changes within the hypovascular tumour circulation produced by a 5 min angiotensin II infusion did not significantly increase tumour 5-FU uptake.

Key words: liver metastasis, regional chemotherapy, fluorouracil, blood flow, angiotensin II
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INTRODUCTION

FORTY PER CENT of patients with large bowel cancer will develop liver metastases [1] and most will be treated by systemic 5-fluorouracil (5-FU) combined with folinic acid which achieves only a 24% partial tumour response [2]. There is a steep dose response relationship between 5-FU concentration and cell kill both *in vitro* [3] and *in vivo* [4], and one explanation for the poor response of liver metastases to chemotherapy may be poor drug penetration into the tumour. This is supported by results with regional infusion of the 5-FU analogue floxuridine, where tumour drug levels are increased approximately 10-fold compared with systemic administration [5], resulting in a 40–50% partial response [6] and significant survival benefit [7]. Further improvement in response to regional floxuridine chemotherapy by increasing the dose or addition of folinic acid has produced unacceptable hepatotoxicity [8] because of a greater first pass drug extraction by normal liver parenchyma than metastases [5]. One difficulty is that colorectal liver metastases are hypovascular resulting in low tumour drug levels [9], while those in normal tissues are high.

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Vasoactive manipulation using agents such as angiotensin II has been proposed as a means of improving tumour blood flow and drug uptake [10, 11]. Previous studies of vasoactive manipulation have been mainly with bolus or very short drug infusions and the findings are not easily extrapolated to the setting of continuous infusion chemotherapy. In addition, the extent to which blood flow and changes in blood flow affect drug uptake is not known.

We have used a dual radiotracer method in a rat liver metastasis model to assess the tumour:liver blood flow ratio and the tumour:liver 5-FU uptake ratio in the same animal. We then compared blood flow and 5-FU uptake ratios after hepatic arterial angiotensin II infusion with those in controls receiving normal saline infusion.

MATERIALS AND METHODS

^{64}Copper (II)-pyruvaldehyde bis(n-4 methyl thiosemicarbazone) (Cu-PTSM)

Cu-PTSM is characterised by efficient extraction from the circulation and prolonged tissue retention. It reacts with intracellular glutathione liberating a copper radionuclide which binds to intracellular macromolecules. Such chemical trapping is an efficient irreversible mechanism giving microsphere-like tracer properties [12]. Tissue concentration of Cu-PTSM may be determined by the relative proportion of cardiac output delivered to the tissues and can be used as a measure of the relative blood flow between different tissues [12, 13]. [^{64}Cu]-labelled Cu-PTSM has been used to measure blood flow in the heart and brain [14] as well as in tumours [12].

Hooded sarcoma-N (HSN) tumour implantation

The HSN tumour is a chemically induced rat sarcoma which is maintained in cell culture by serial passage, and develops with similar hypovascular characteristics to human colorectal liver metastases [15, 16]. Intraportal injection of an HSN cell suspension produces a median of five [interquartile range (IQR) 2–9] intrahepatic tumours in 80% of animals by between 22 and 28 days from implantation. Male CBH/cbi rats (weight range 300–350 g) were anaesthetised by 1–2% halothane inhalation and a short lower midline abdominal incision was made. Between 500 and 800 HSN cells were then injected into a portal vein tributary, the abdominal wall sutured, and the animals allowed to recover.

[^{64}Cu]PTSM, [^{3}H]5-FU and angiotensin II preparation

'No carrier' added ^{64}Cu ($t_{1/2}$ = 12.7 h) was used to label the ligand H_2-PTSM as previously described [12]. Cu-PTSM was prepared by buffering an aqueous solution of $^{64}CuCl_2$ in 0.3 M HCl with two equivalents of 3 M sodium acetate (pH 4.6). To this was added an ethanolic solution of H_2-PTSM ligand (0.1 μg/μl). The radiochemical yield averaged 94% and the octanol water partition coefficient (log P) of this labelled product was on average 1.6. The final Cu-PTSM solution was diluted with 0.9% NaCl to a radioactive concentration of 200–400 μCi/ml (7.4–14.8 MBq/ml) containing 5% ethanol. 5-[6-^{3}H]-fluorouracil ([^{3}H]5-FU) was obtained commercially (NEN Dupont, Dreich, Germany), and used at a concentration of 1 mCi/ml (8.7 μg/ml). A 40 μCi dose was administered to each animal over a 5 min period representing a total dose of 348 ng of 5-FU at an estimated circulating concentration of 70 ng/ml within the tumour-bearing liver. This 5-FU concentration is of the same order as is obtained clinically with systemic 5-FU infusion, and previous studies in the HSN liver metastasis model using angiotensin II [17] or vasopressin [18] infusion have suggested that the maximum enhancement of tumour:liver uptake occurs within the first 5 min of infusion. Angiotension II (Sigma, Poole, U.K.) was prepared freshly from frozen aliquots of stock solution (100 μg/100 μl) and made up to the required volume with 0.9% NaCl. The dose used was higher than that used in previous studies of regionally infused angiotensin II [19] and was based on a previous study [17] of the dynamics of the angiotensin II response using laser Doppler flowmetry.

Experimental sequence

Animals were anaesthetised by inhalation of 1–2% halothane. Via midline laparotomy, the lesser omentum was dissected to expose the gastroduodenal artery which is a side branch of the hepatic artery. The gastroduodenal artery was then cannulated with a polyethylene cannula (Portex Ltd, Hythe, U.K.; 0.28 × 0.61 mm, i.d. × o.d.) primed with heparinised saline which was subsequently used to administer infusions directly into the hepatic arterial circulation. A 30 min infusion of either normal saline (25 μl/min, $n = 20$ animals) or angiotensin II (1 μg/min at 25 μl/min, $n = 10$ animals) into the hepatic arterial circulation was commenced via the gastroduodenal artery. The angiotensin II infusion time was chosen to allow a period of stabilisation following the initial vasoactive changes, to produce a model which resembled infusion therapy more than bolus administration. Longer infusion periods had to be avoided because of the cardiovascular effects associated with prolonged anaesthesia and laparotomy.

The Cu-PTSM and the [^{3}H]5-FU were administered simultaneously at a flow rate of 25 μl/min using an infusion pump via the side arm of a T-piece, for a 5 min period starting at 25 min after onset of the angiotensin or saline infusion. Higher infusion rates were found to increase the likelihood of retrograde flow within the hepatic artery resulting in systemic circulation spillover. After the end of the tracer infusions 5 min were allowed to elapse to ensure maximum uptake from the circulation before the animals were killed by potassium chloride injection. Liver and metastases were then separated for weighing and counting.

Sample preparation and counting

Tumours were individually dissected from the liver, weighed and placed in counting vials. The liver was divided into blocks of approximately 800 μg which were weighed prior to counting. All liver tissue was included and prepared for counting. ^{64}Cu activity in each hepatic lobe and tumour was counted using a gamma counter with decay and background correction factors applied. Blocks were left in sample tubes at room temperature for at least one week to allow the ^{64}Cu activity to decay and were then prepared for tritium liquid scintillation counting to assess [^{3}H]5-FU uptake. Each block was further subdivided into <200 μg pieces which were then transferred to individual scintillation counting vials to ensure that the entire contents had been transferred. Blocks were individually homogenised, solubilised using a 50:50 mixture of soluene 350 (Packard) and isopropyl alcohol for 12 h at

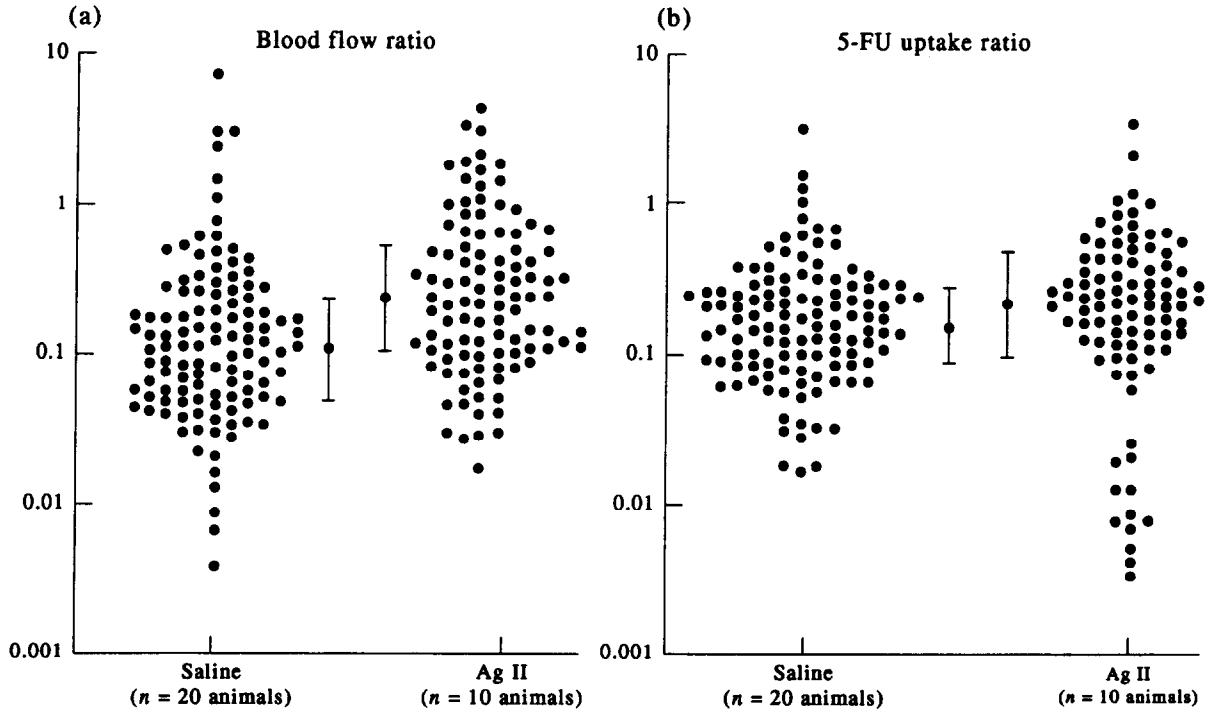

Figure 1. Cu-PTSM and [^{3}H]5-FU tumour:liver uptake ratios (vertical axes). There was a wide variation (×1000-fold) in tumour:liver blood flow and 5-FU uptake ratios (vertical axes plotted to a log scale) which was not explained by individual tumour size or extent of liver metastases within an individual animal. Angiotensin II produced a significant increase (nested design analysis of variance, $P = 0.05$) in blood flow but not 5-FU uptake ratio.

35°C, and bleached with 0.5 ml hydrogen peroxide (30% v/v, Sigma), after which 10 ml scintillant was added (Hi-Ionic Fluor, Packard). Luminescence was allowed to subside for 24 h before counting each block for 1 min in a liquid scintillation counter.

Statistical methods

Tumour:liver radioactive count ratios for both isotopes were calculated for each tumour based on the total counts/g for each tumour divided by the counts/g of the liver lobe in which the tumour was situated. The effect of angiotensin infusion on tumour:liver blood flow and 5-FU uptake ratios was assessed by nested design analysis of variance [20] using log-transformed data. This suggested a significant within animal covariance in tumour:liver flow ($P < 0.001$) and 5-FU uptake ($P < 0.001$) ratios. Therefore, Pearson correlations comparing flow and 5-FU uptake were performed using a single data point for each animal derived by obtaining the median of all tumour:liver ratios in that animal. These data were plotted on a log scale to clarify presentation of the figures. The percentage hepatic replacement (PHR) was derived by expressing the quotient of the total tumour weight (g) by the total tumour and liver weight (g) in an individual rat as a percentage.

RESULTS

The overall median PHR by tumour was 6.7% (IQR 2.2–11.4). There was no significant difference (Mann–Whitney U test, $P = 0.16$) in PHR between the saline and angiotensin II groups.

There was wide (>1000-fold) variation in Cu-PTSM tumour:liver uptake ratios between individual tumours when saline was infused (Figure 1) which did not significantly correlate with tumour weight ($r = -0.06$, $P = 0.52$) or PHR ($r = -0.20$, $P = 0.4$).

There was a significant correlation ($r = 0.957$, $P < 0.0001$) between tumour:liver Cu-PTSM and tumour:liver [^{3}H]-5-FU count ratios in the saline (Figure 2) but not the angiotensin II ($r = 0.51$, $P = 0.13$) group.

Comparison of tumour:liver ratios with either saline or angiotensin II using nested analysis of variance suggested that angiotensin II significantly increased tumour:liver flow ($P = 0.05$) but not 5-FU ($P = 0.29$) ratio.

DISCUSSION

The tumour model we used in this study was selected because it resembles colorectal liver metastases in being hypovascular. We found a wide (1000-fold) variation in tumour:liver blood flow ratios in this model (Figure 1). The lack of correlation between tumour:liver blood flow ratio and tumour size or PHR suggests that the variation observed was not due to hypovascular necrotic areas within larger tumours or to changes in the hepatic perfusion index produced by increasing tumour burden [15].

We found a close correlation between tumour:liver blood flow ratio and tumour:liver 5-FU uptake ratio with saline infusion (Figure 2). This relationship between blood flow and drug uptake has not been demonstrated before. Regional angiotensin II infusion resulted in a modest (× 2) but significant increase in tumour:liver blood flow ratio (Figure 1).

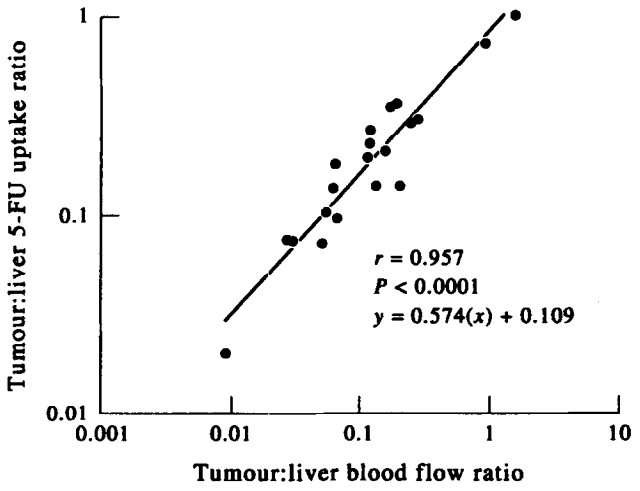

Figure 2. Correlation between tumour:liver Cu-PTSM and tumour:liver [^{3}H]5-FU count ratios with saline infusion. There was a close relationship ($r = 0.957$, $P < 0.0001$) between tumour:liver blood flow and 5-FU uptake ratio in animals receiving saline infusion. Each point represents the median of all tumour:normal ratios for a particular animal. The axes have been plotted to log scales.

Previous reports of hepatic tumour blood flow manipulation with vasopressor agents have also reported an increase in tumour:liver blood flow ratio [19, 21]. We have previously shown [17], using laser Doppler flowmetry, that there is an absolute fall in liver and tumour perfusion in response to angiotensin II infusion. This suggests a proportionately greater vasoconstriction of normal liver parenchyma than tumour in response to angiotensin II, resulting in a net increase in tumour:liver blood flow ratio.

The small angiotensin-induced increase in 5-FU uptake ratio (Figure 1) was less than that which would be predicted from the relationship between flow and 5-FU uptake ratios recorded in the saline infusion group (Figure 2). Despite an increased 5-FU blood-concentration arising from angiotensin-induced reduction in blood flow [17] at constant 5-FU infusion dose, 5-FU uptake did not reproduce the blood flow increase observed with Cu-PTSM and there ceased to be a significant correlation between tumour:liver blood flow and 5-FU uptake ratios. Other factors, such as 5-FU interstitial diffusion away from tumour vessels [22], may have prevented the comparable increase in tumour 5-FU uptake.

Thus, the limited vasoactive changes within the hypovascular tumour circulation which it was possible to achieve with angiotensin II in this study did not increase tumour 5-FU uptake. Therapeutically, effective increases in tumour 5-FU levels may require both absolute increases in tumour blood flow and perhaps also shorter diffusion distances between the vessels carrying increased flow.

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