Noradrenaline improves the tumour to normal blood flow ratio and drug delivery in a model of liver metastases

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Background: Vasopressors administered via the hepatic artery appear to increase drug delivery to colorectal liver metastases, but are limited by a short duration of action. This study measured their effect on blood flow and drug delivery during a prolonged infusion in a model of liver metastases.

Methods: In Hooded Lister rats with liver metastases, blood flow in tumour and adjacent normal liver was measured using laser Doppler flowmetry during a 30-min hepatic arterial infusion of endothelin 1, angiotensin II, vasopressin, N-nitro-L-arginine methyl ester (L-NAME), noradrenaline or saline (n = 6 per group). The same agents were co-administered with radiolabelled 5-fluorouracil (5-FU) (n = 6 per group) and uptake in the tumour and normal liver was measured.

Results: The mean(s.d.) duration of effect and resulting percentage changes in tumour to normal blood flow ratio of the vasopressors during this period were: noradrenaline, 2.9(0.4) min and 34(5) per cent (P < 0.05); angiotensin II, 4.2(0.2) min and 10(2) per cent (P < 0.05); vasopressin, 11.1(0.9) min and 7(2) per cent (P < 0.05); endothelin 1, 21.5(2.3) min and 14(5) per cent (P < 0.05); and L-NAME, 22.6(3.3) min and 2(1) per cent (P not significant). The mean(s.d.) uptake of radiolabelled 5-FU by the tumour in the groups studied was: saline, 5.1(3.2) × 10^5 c.p.m. per g tissue; angiotensin II, 5.1(1.4) × 10^5 c.p.m. per g; endothelin 1, 15.8(4.2) × 10^5 c.p.m. per g; L-NAME, 3.5(1.3) × 10^5 c.p.m. per g; and vasopressin, 6.8(3.5) × 10^5 c.p.m. per g. Significant improvements in 5-FU uptake only resulted from noradrenaline infusion (22.0(9.8) × 10^5 c.p.m. per g; P < 0.05).

Conclusion: These findings suggest that hepatic arterially infused noradrenaline might be used to improve drug delivery to liver metastases.

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Introduction

Hepatic arterial chemotherapy produces a higher loco-regional response rate than conventional systemic chemotherapy in patients with colorectal liver metastases. Initial clinical studies suggest this may translate into improved survival^1,2 and enhanced quality of life^3. The rationale behind this route of therapy is that liver metastases receive up to 95 per cent of their blood supply via the hepatic artery, so a greater proportion of a chemotherapeutic agent will be delivered to the tumour.

Further improvements in drug delivery might be achieved by a greater understanding of the differences that exist between tumour and normal hepatic vessels. Using electron microscopy it is apparent that blood vessels within colorectal liver metastases lack both innervation and a developed smooth muscle coat^4. As such these ‘tumour’ blood vessels are unlikely to possess any significant neurogenic vasoconstrictor capability.

Appreciation of this observation may allow selective vasoconstriction of normal hepatic vessels while leaving tumour vessels unaffected. This in turn should lead to shunting of blood into the tumour with a concomitant improvement in drug delivery. Regionally infused vasopressors improve the uptake of chemotherapeutic agents by liver metastases presumably by enhancing tumour blood flow^5. However, their short duration of action is a limiting factor.

In order to assess the mechanism and efficacy of regionally infused vasopressors, an animal model of
colorectal liver metastases, in which tumour blood vessels have a similar anatomical and physiological profile to those in human colorectal liver metastases\(^6\),\(^7\), has been developed.

### Materials and methods

Experiments were performed using male Hooded Lister rats (350–400 g) which 14 days previously had undergone intraportal inoculation of \(1 \times 10^6\) MC28 syngeneic tumour cells. This produced between two and five tumours per rat measuring from 5 to 8 mm on the liver surface. Seventy-two animals then underwent laparotomy under halothane anaesthesia and the gastroduodenal artery was cannulated with fine-bore tubing (Portex UK, Hythe, UK) using an operating microscope\(^8\).

All animal work was carried out under Home Office approval.

### Blood flow measurements

Microcirculatory blood flow was measured using laser Doppler flowmetry (MBF3D; Moor instruments, Axminster, UK). The probe design allows measurement up to a depth of 2 mm and the flow values are expressed in arbitrary flux units which are proportional to microcirculatory blood flow. Before each experiment the probe was recalibrated and a time constant of 3-0 s at 0-25 Hz was used to minimize movement artefact.

A probe measuring 30 \(\times\) 1 mm was placed over the centre of the tumour in a specially designed holder and another was placed on an area of adjacent normal liver within the same lobe but 2 cm away. To minimize pressure from the holder and probe the wire was suspended from a clamp. The laparotomy wounds were closed to prevent drying of the liver surface and the core temperature was maintained using a heated mat.

Each rat was infused for 30 min with one of the vasopressors listed below and baseline measurements were taken for 5 min before and after infusion.

All data were collected and analysed using the Maclab PC data logging system (Apple, Cupertino, California, USA). The duration of effect and flow changes, as demonstrated by the areas under the flux line curves, were calculated.

### Regionally infused vasoconstrictors

Thirty-six rats were divided into six groups (\(n = 6\) in each) and each group received a different agent. Agents were prepared in 0-9 per cent saline before each experiment and kept at rat core temperature before infusion. The dose of agent chosen was that which produced a 20 per cent rise in systemic blood pressure. Agents were infused over 30 min (in a volume of 1 ml) using an infusion pump (Harvard, Cambridge, Massachusetts, USA) into the hepatic artery via the gastroduodenal artery.

The agents used were: \(N\)-nitro-L-arginine methyl ester (\(L\)-NAME) 1-5 mg per kg per min, noradrenaline 5 \(\mu\) g per kg per min, vasopressin 1 \(\mu\) g per kg per min, endothelin 1 0-5 \(\mu\) g per kg per min, angiotensin II 0-35 \(\mu\) g per kg per min and saline in the control group. All vasoactive agents were purchased from Sigma (Poole, UK).

### Radiolabelled 5-fluorouracil

Another group of rats (\(n = 36\)), 14 days after intraportal inoculation of MC28 tumour, underwent cannulation of the gastroduodenal artery. Identical doses of the same vasopressors in conjunction with \(^{[\text{H}]5}\)-fluorouracil (5-FU) were infused in 1 ml over 30 min (\(n = 6\) in each group). At the end of this period the animals were killed and the livers were removed.

Tumour and liver from the same lobe (taken 2 cm away from the tumour) were removed and weighed. The tissue was solubilized and diluted in scintillant (Toluene Scintillator; Packard, Reading, UK). Samples were radio-counted and values expressed as counts per gram of tissue per minute.

### Statistical analysis

The data underwent one-way analysis of variance followed by unpaired \(t\) test corrected for unequal variances. A probability of \(P < 0\text{.}05\) was considered significant.

### Results

#### Laser Doppler flow

The tumour to normal blood flow ratio (T:N) before commencing the infusions ranged between 0-25 and 0-35 (mean 0-30) which is similar to that found in human colorectal liver metastases.

All vasopressors studied produced a variable drop in flux measurement in both tumour and normal liver; all agents apart from \(L\)-NAME produced almost immediate effects and the time course in both tumour and liver was equal. None of the agents produced an absolute rise in tumour blood flow.

The percentage changes in the T:N ratio during the infusion periods can be considered in two ways. First, when taken over the whole 30-min infusion period, none of the agents produced a significant change. However, if the period in which the agent had its effect is considered
separately (as defined as the period taken for the flux lines to return to the original preinfusion point) the mean(s.d.) percentage change in T : N ratio and duration of action were as follows: angiotensin II, 10(2) per cent (\(P < 0.05\)) and 4\(\pm\)2(0\(\pm\)2) min; noradrenaline, 34(5) per cent (\(P < 0.05\)) and 2\(\pm\)9(0\(\pm\)4) min; vasopressin, 7(2) per cent (\(P < 0.05\)) and 11\(\pm\)1(0\(\pm\)9) min; endothelin 1, 14(5) per cent (\(P < 0.05\)) and 21\(\pm\)5(2\(\pm\)3) min; and LL-NAME, 3\(\pm\)5(1\(\pm\)3) per cent (\(P < 0.05\)) and 22\(\pm\)6(3\(\pm\)3) min. Changes in the T : N ratio are illustrated in Fig. 1.

\[^3\text{H}]\text{5-fluorouracil uptake}\]

None of the agents produced a significant difference in the uptake of \[^3\text{H}\]5-FU in normal liver compared with the saline group (Fig. 2). Mean(s.d.) uptake into the tumour was as follows: saline, 5\(\times\)1(3\(\times\)2) \(\times\)10\(^5\) c.p.m. per g tissue; angiotensin II, 5\(\times\)1(1\(\times\)4) \(\times\)10\(^5\) c.p.m. per g; endothelin 1, 15\(\times\)8(1\(\times\)4) \(\times\)10\(^5\) c.p.m. per g; L-NAME, 3\(\times\)5(1\(\times\)3) \(\times\)10\(^5\) c.p.m. per g; noradrenaline, 22\(\times\)0(9\(\times\)8) \(\times\)10\(^5\) c.p.m. per g; and vasopressin, 6\(\times\)8(3\(\times\)5) \(\times\)10\(^5\) c.p.m. per g. Noradrenaline was the only agent to produce a significant increase in radiolabelled 5-FU uptake in the tumour (\(P < 0.05\)) as illustrated in Fig. 3.
Differences between blood vessels in tumour and normal hepatic tissue were first suggested in 1969. More recently, using electron microscopy and immunohistochemistry, blood vessels in colorectal liver metastases were shown to lack both a complete smooth muscle wall and neuronal innervation. A similar profile has been demonstrated in the blood vessels supplying MC28 tumours, suggesting a comparable situation to that in humans.

These findings lend support to the hypothesis that selective vasoconstriction of normal, but not tumour, hepatic vessels achieved by the administration of regionally infused vasopressors would result in blood being shunted from the hepatic vascular bed into the tumour and hence improve drug delivery.

Adrenaline has long been known to improve the tumour blush in hepatic angiography of colorectal liver metastases, beyond that which might be expected from vasconstriction of normal surrounding vessels alone. Noradrenaline has been shown to produce increased uptake of radiolabelled microspheres when co-administered in conjunction with propanolol via the hepatic artery in an animal model, although its duration of action was limited. Ackerman et al. demonstrated increased capillary blood flow, using laser Doppler flowmetry, within intrahepatic tumours due to infused catecholamines in an animal model. Goldberg and colleagues demonstrated improved uptake of radioactive tracers with angiotensin II in patients with colorectal liver metastases receiving hepatic arterial chemotherapy. Use of intraoperative laser Doppler in patients with colorectal liver metastases has demonstrated improved tumour blood flow when angiotensin II was administered via the hepatic artery. It has been suggested that part of the effect is due to the increased systemic blood pressure and hence increased tumour perfusion pressure, producing a subsequent opening up of tumour vessels. This is unlikely, however, as the changes in pressure do not mirror the changes in tumour blood flow demonstrated. Dworkin et al., using an animal model, demonstrated improvements in the T:N ratio of intrahepatic tumours using vasopressin with and without a nitric oxide synthase inhibitor. The results of this study suggest that addition of such inhibitors prolongs the effect of vasopressin and implicates nitric oxide production within hepatic vessels as a possible cause of the limited duration of action seen with hepatic arterially infused vasopressors. Using the same animal model, Dworkin et al. demonstrated that although hepatic arterially infused angiotensin II led to improvement in the T:N ratio it did not lead to a significant improvement in drug uptake. A similar study demonstrated that only endothelin 1 produced a significant improvement in the T:N ratio when infused over 30 min.

One of the limitations of most studies to date is that vasopressors have been given as either a bolus or short infusion. Given that hepatic arterial chemotherapy is administered over a prolonged period, assessment of the action of regionally infused vasopressors over this time course would be required before contemplating a clinical trial.

This study confirms the limited duration of action of vasopressors seen previously and illustrates their ability to raise the T:N ratio. The reduction in blood flow seen in the tumour circulation (although less than that in the liver) might be due to constriction of feeding vessels coming from the normal adjacent liver or might represent an element of tumour vessel constriction.

Downregulation of sympathetic innervation in tumour vessels might explain why noradrenaline (a sympathetic neurotransmitter) produced the greatest rise in the T:N ratio. If noradrenaline receptors were downregulated to a greater extent than those for other neurotransmitters then its administration would lead to less intrinsic tumour vessel constriction and hence produce a greater increase in the T:N ratio.

Other groups have demonstrated improved drug uptake and blood flow in hepatic tumours using endothelin 1, angiotensin II and vasopressin. The animal studies involving these agents used different tumours to the one employed in this study which may account for the different results reported here. It should be noted that the blood vessels found within MC28 tumours possess a similar profile with regard to both structure and innervation to those found in human colorectal liver metastases. The dose used is relevant when comparing the results of this study with those from similar investigations in animal and humans. Many of these studies used higher doses of vasopressors than described here, and produced sustained marked hypertension (greater than the 20 per cent level in this study). Such effects would be unacceptable in a clinical setting. These variations in doses may account for the greater effectiveness noted for agents such as angiotensin II in similar reported studies.

As none of the agents produced improvements in tumour blood flow in real terms it is surprising that noradrenaline caused an increased uptake of radiolabelled 5-FU in the tumours. Jain demonstrated that such tumours exist in a state of interstitial hypertension, which retards the movement of molecules such as chemotherapeutic agents through the interstitium. This interstitial hypertension leads to radially outward convection within...
the tumour periphery which opposes inward diffusion. Part of the effect produced by these vasopressors might be a reversal of the pressure gradient around the tumour leading to improved drug delivery. Since noradrenaline produced the greatest change in the T:N ratio it may be the only agent to have passed the threshold required to reverse the gradient. If the main action is achieved through pressure changes rather than shunting, then this may explain why uptake in the normal liver was unaffected.

Vasopressors increase the T:N ratio and noradrenaline specifically improves drug delivery when co-administered with hepatic arterial chemotherapy. However, as hepatic arterial chemotherapy is usually given as a prolonged infusion the limited duration of action of the vasopressors studied may preclude their use in a clinical setting. A greater understanding of their pharmacokinetic profile, especially with regard to the refractory period, may allow incorporation of these agents into a regional chemotherapeutic trial.

References