

# Temporary blood flow stasis with degradable starch microspheres (DSM) for liver metastases in a rat model

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**SUMMARY** A reliable liver metastasis model using intraportal injections of sarcoma cells was established in syngeneic hooded Lister rats to study the blood supply of the tumours and to evaluate the role of degradable starch microspheres (DSM) in conjunction with selective hepatic arterial and portal venous chemotherapy. The tumour/normal liver (T/L) ratio after intra-arterial and intraportal injection of  $^{113}\text{Sn}$  microspheres was 1.04 (range: 0.38–1.15) and 0.03 (range: 0.006–0.22), respectively. After intravenous  $^{14}\text{C}$ -iodoantipyrine quantitative autoradiography of tumour and normal regions demonstrated a mean T/L ratio of  $0.74 \pm 0.05$ . After hepatic artery ligation (HAL) and portal vein ligation (PVL) the values were  $0.32 \pm 0.05$  and  $0.42 \pm 0.05$ , respectively. These results confirm that the vascularity of the tumours in this model is similar to human colorectal cancer metastases. Radiolabelled  $^{14}\text{C}$  5-Fluorouracil (5-FU) was given intravenously, *via* the hepatic artery and *via* the portal vein (the latter two routes with and without DSM). Quantitative autoradiography of tumour regions showed that selective hepatic arterial administration with DSM resulted in a significantly increased concentration of  $^{14}\text{C}$ -5-FU within the tumours. These results suggest that DSM may enhance the therapeutic benefit of hepatic arterial 5-FU by increasing its uptake into tumours.

The treatment of multiple colorectal liver metastases has been a subject of controversy for the last 30 years. Surgical removal of resectable solitary metastases and symptomatic palliation of unresectable tumours is justified in selected patients.<sup>1</sup> The role of chemotherapy for multiple metastases has been limited although there has been a resurgence of interest because of the innovation of implantable systems which enable constant longterm infusion to be given on an outpatient basis.<sup>2</sup> In spite of the development of new cytotoxic drugs and various multiple regimens with more established agents, none have been found to be superior to the single agent 5-Fluorouracil (5-FU) or its derivative fluorodeoxyuridine (FUDR). A more recent concept is the combination of degradable microspheres and cytotoxic drugs which can be selectively infused into the hepatic artery. The micro-

spheres are trapped in the tumour and the drug which is incorporated within the substance of the microsphere is then slowly released as the microsphere disintegrates allowing prolonged exposure of the tumour to the cytotoxic agent.<sup>3</sup> Alternatively, the drug can be mixed in solution with the degradable starch microspheres (DSM) which, after infusion, are trapped in the tumour. Hopefully the blood flow stasis which follows, allows increased concentrations of drug to diffuse into the tumours.<sup>4</sup>

The use of DSM results in reduced systemic concentrations of cytotoxic drugs after selective hepatic arterial administration.<sup>5,6</sup> It is not known, however, whether the concentration of the drug is increased within the tumours themselves. Recent studies have indicated that liver metastases possess enzymes required for breakdown of 5-FU into its active metabolites.<sup>7</sup> Accordingly, accurate assessment of any technique capable of increasing uptake of 5-FU into metastatic liver tissue deserves assessment. This is difficult in man but can be accurately

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measured with the use of an animal model provided the liver tumours have a similar blood supply to that of colorectal liver metastases.

## Methods

### ANIMALS

A liver metastasis model was established in the rat to study both blood flow and uptake of cytotoxic agents in tumours following injection by different routes.

Initial studies were done to establish the time necessary for the optimum production of liver metastases in the rat following mesenteric vein injection of tumour cells.

A laparotomy was carried out on 40 anaesthetised Hooded Lister rats weighing 160–250 g. The small bowel and caecum were laid out to expose the adjacent mesentery. A suspension of  $10^6$  sarcoma cells (MC 28) was injected slowly in a volume of 0.3 ml into a peripheral tributary feeding into the mesenteric vein. The abdominal wall was closed and the rats allowed to recover. They were maintained on a routine diet. Groups of rats were killed between 12 and 24 days after injection. The livers were removed and all macroscopic tumours excised and weighed separately from the normal liver.

### BLOOD SUPPLY OF LIVER TUMOURS

A group of 34 rats was used to study the blood supply of established liver tumours by two different methods. They were studied on days 16 and 17 after intraportal injection of tumour cells.

#### *Radioactive microspheres*

Intra-arterial ( $n=9$ ) and intraportal ( $n=11$ ) injections of  $^{113}\text{Sn}$  microspheres ( $10^5$ , 15  $\mu$ ) were given, using ether general anaesthesia, to determine the relative distribution of blood flow to normal liver and the tumours. The intra-arterial injections were given with the tip of a cannula placed in the upper descending aorta *via* the left carotid artery. The intraportal injections were given into a peripheral tributary of the mesenteric vein. The rats were killed immediately after the procedure, the livers removed and the tumours excised from the hepatic parenchyma. The tumours and normal liver were separately weighed and placed into universal containers to determine the respective counts of radioactivity/min using a gamma well-scintillation counter (Kontron Gammatic I). On account of the wide variations of tumour mass between rats, the counts of activity were corrected to unit gram of tumour or normal liver tissue. These values were used to derive a tumour/normal liver (T/L) ratio which is an index of the relative distribution of the hepatic arterial and portal venous blood to the tumours.

#### *Quantitative autoradiography*

An index of tumour perfusion was measured by quantitative autoradiography in 12 rats with multiple liver tumours after a controlled infusion of a highly diffusible tracer,  $^{14}\text{C}$ -iodoantipyrine, under ether general anaesthesia. The infusion was given through a cannula placed in the femoral vein for exactly 60 seconds. Four rats underwent laparotomy alone, four underwent portal vein ligation (PVL) and four had hepatic artery ligation (HAL) immediately before the infusion so that the overall relative tumour flow and selective arterial and portal components could be determined. The rats were killed instantly by decapitation at 60 seconds. The livers were rapidly removed, cut into suitable blocks containing tumour/s and adjacent normal liver and immersed in isopentane chilled to  $-60^\circ\text{C}$  with liquid nitrogen. Four to six alternate 20  $\mu$  sections were cut from the blocks using a cryostat, placed on cover slips and fixed by rapid warming to  $75^\circ\text{C}$  for five minutes. Alternate sections were taken for histological verification of the location of small tumours. Sections of 41 tumours with adjacent normal liver and a series of standards containing known concentrations of  $^{14}\text{C}$ -methyl methacrylate from 400 to 1000  $\mu\text{Ci}$  were exposed to standard radiograph films for two weeks in light tight cassettes. The films were developed and the densitometer<sup>9</sup> was calibrated with the standards in order that the concentration of  $^{14}\text{C}$ -iodoantipyrine within the sections could be extrapolated from the optical density of the films. The optical density of 0.1  $\text{mm}^2$  areas, corresponding to tumour and normal liver regions, were measured from four to six alternate sections from which a mean concentration of  $^{14}\text{C}$ -iodoantipyrine was derived. These concentrations were corrected from the respective partition coefficients of tumour and normal liver tissue and expressed as a T/L ratio.

#### *Tissue: blood partition coefficients*

The partition coefficients of normal liver and tumour tissue for  $^{14}\text{C}$ -iodoantipyrine were determined from seven different samples of normal liver and six tumours of two rats, killed 16 days after injection of tumour cells. The concentration of the tracer was measured in the blood, normal liver and tumour tissue after a suitable equilibration period. The tracer is known to equilibrate with most tissues of the body in less than 30 seconds. As antipyrine is degraded by the liver at a rate of 6% per hour, 10 minutes were allowed to elapse after an intravenous injection of  $^{14}\text{C}$ -iodoantipyrine before the rats were killed. Blood samples were taken from the severed neck and the concentration of radioactivity measured in a liquid scintillation counter. Autoradiograms of the sections containing tumours were used to measure the con-

concentration of tracer in normal and tumour tissue by densitometry. A ratio of the tissue/blood concentration gave the partition coefficient for the tumour and normal liver tissue, respectively.

#### UPTAKE OF 5-FU BY TUMOURS

A measured quantity of  $^{14}\text{C}[2]$ -5-FU was given with and without DSM (45 u, 'Spherex', Pharmacia Ltd) to 30 rats with multiple liver tumours 16 or 17 days after the injection of  $10^6$  cells into the mesenteric vein. The radiolabelled drug was given *via* one of three routes: (1) systemically into a peripheral vein; (2) into the hepatic artery, with and without  $10^6$  DSM, through a cannula inserted into the gastroduodenal artery with the aid of an operating microscope and (3) into a peripheral tributary of the portal vein with and without  $10^6$  DSM (the Table shows number of rats and tumours studied). The intravenous and intraportal injections usually took about 30 seconds, whereas, the hepatic arterial injections with DSM took up to three minutes to minimise retrograde flow and loss of 5-FU to other organs – for example, spleen. The rats were killed five minutes after the start of the injections and the same method of processing and quantitative autoradiography was used as for the  $^{14}\text{C}$ -iodoantipyrine blood flow studies described above.

The concentration of  $^{14}\text{C}$  from representative areas of the tumours was determined by densitometry. To enable values of  $^{14}\text{C}$  to be obtained within the reference range of the standards used for quantitative densitometry, the dose of 5-FU was standardised to 20 mg for each animal. The known concentration of  $^{14}\text{C}/\text{mg}$  5-FU enabled the concentration of the isotope to be interpreted as that of 5-FU or its metabolites within the tumours.

## Results

#### LIVER 'METASTASIS' MODEL

The mean weights of the excised liver tumours from each group of rats killed on different days are shown in Figure 1. The tumours were extremely small on day 12 and 13 followed by apparent exponential growth which levelled off after day 20. The tumours became progressively more necrotic and difficult to excise from normal liver during the latter part of the

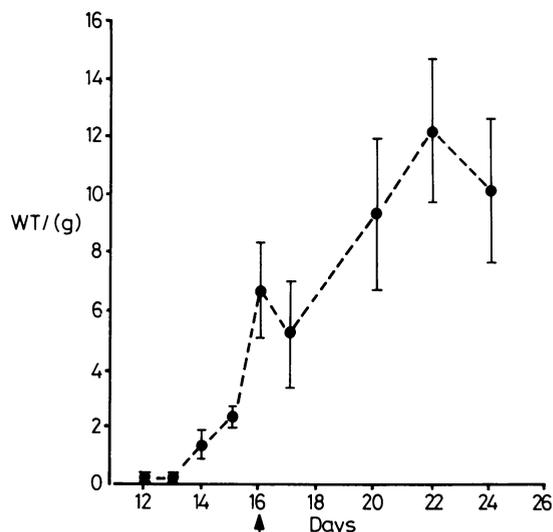


Fig. 1 Mean tumour weights ( $\pm$ SD) on different days of death after intraportal injection of  $10^6$  MC-28 sarcoma cells. Day 16 (arrowed) was the most suitable day for the 'metastasis' model.

study and the general condition of the rats rapidly deteriorated. It was found that the tumours were most suitable for further study at day 16 when the mean weight was  $6.65 \pm 1.5$  (SD). The tumours were only occasionally too large and necrotic at this time and there was seldom extrahepatic involvement in the portal region. The actual number of tumours varied considerably between individual rats which resulted in different numbers used in the 5-FU studies.

#### BLOOD FLOW STUDIES

The distribution of T/L ratios after intra-arterial and intraportal injection of  $^{113}\text{Sn}$  microspheres are shown in Figure 2. The two groups are widely separated with the arterial and portal groups having a median value of 1.04 (range: 0.38–1.15) and 0.023 (range: 0.006–0.22), respectively. The blood supply is extrapolated from the relative distribution of microspheres to tumour and normal liver. Thus, overall tumour tissue has an arterial blood supply similar to normal liver on a weight for weight basis, whereas, only a minimal fraction of overall portal supply to the liver feeds the tumours.

The  $^{14}\text{C}$ -iodoantipyrine studies complement the microsphere results as they are an index of actual tissue perfusion. The partition coefficients of tumour and normal liver tissue for  $^{14}\text{C}$ -iodoantipyrine were  $0.46 \pm 0.03$  (SD) and  $0.83 \pm 0.02$ , respectively. The individual T/L values are illustrated in Figure 3. The mean T/L ratio of the control group of rats was 0.7

Table Number of rats and tumours in  $^{14}\text{C}$ -5-FU studies DSM was mixed with the drug in two groups through the hepatic artery and portal vein

Route	IVI	HAI	HAI + DSM	PVI	PVI + DSM
Rats	4	6	9	7	4
Tumours	20	23	37	39	38

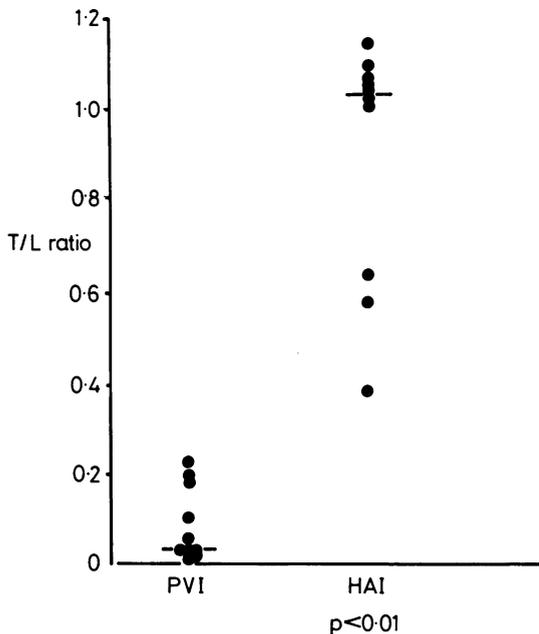


Fig. 2 Tumour/liver ratio after intraportal injection (PVI) and intra-arterial (HAI) injection of  $^{113}\text{Sn}$  microspheres.

( $\text{SE} \pm 0.05$ ) which suggests that the tumours are less vascular than normal liver. Relative tumour perfusion is likely to be even less, however, as preportal

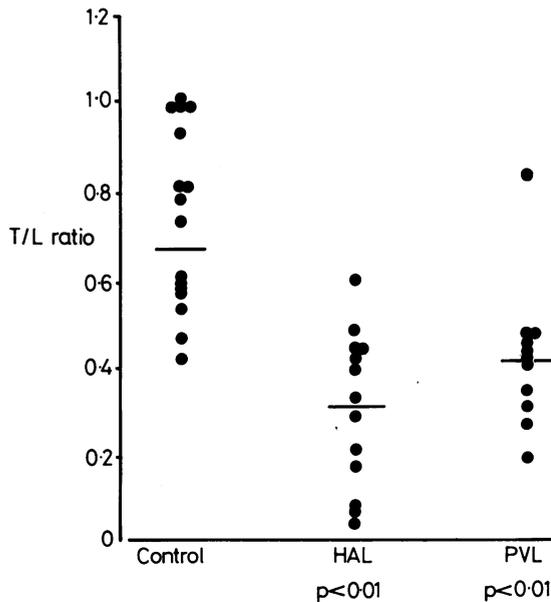


Fig. 3 Tumour/liver ratio after  $\text{iv } ^{14}\text{C}$ -iodoantipyrine in rats which underwent laparotomy only (control), those which had hepatic artery ligation (HAL) and portal vein ligation (PVL).

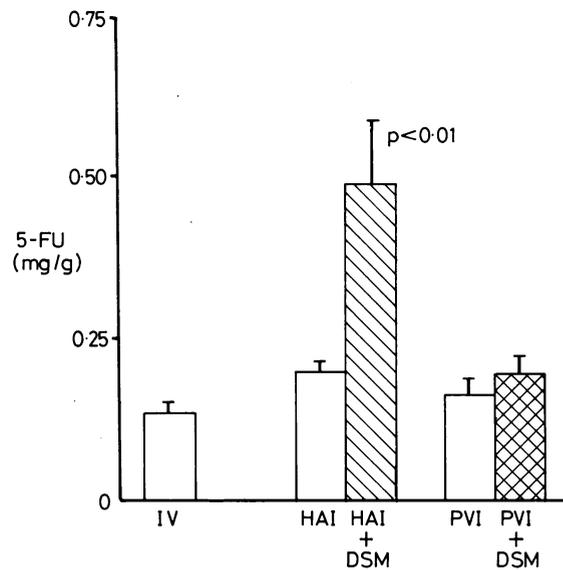


Fig. 4 Uptake of  $^{14}\text{C}$ -5-FU in tumours having been given intravenously (IVI), into the hepatic artery (HAI) and into the portal vein (PVI) with and without DSM.

organs would have extracted the tracer that would have been destined for the liver arriving by the portal vein. After HAL and PVL the mean T/L ratio of the respective 13 and 11 tumours was 0.32 ( $\text{SE} \pm 0.05$ ) and 0.42 ( $\text{SE} \pm 0.05$ ). Although both these results are significantly less than the control group,  $p < 0.01$  – Duncan's multiple range test,<sup>10</sup> they are not directly comparable owing to inconsistent consequences of preportal extraction of the tracer. The results of HAL and PVL, however, may be compared with each other and the microsphere studies. Ligation of the portal vein eliminates the error of preportal extraction and the T/L ratio is half that derived from the microsphere technique, suggesting there is preferential flow to normal liver when the liver is totally dependent on the hepatic artery for a blood supply. After HAL, a significant fraction of portal venous flow supplies the tumours.

#### 5-FU UPTAKE STUDIES

The concentration of isotope ( $^{14}\text{C}$ ) within representative areas of the tumours was measured and with the known amount of 5-FU and radioactivity administered to each rat, the results were expressed as 5-FU mg/g tumour tissue. The mean values for the five groups are shown in Figure 4. They show a significantly higher concentration of 5-FU (or its metabolites) within the tumours after hepatic arterial injection with DSM compared with all the other routes of administration. Autoradiograms of liver sections containing tumours show increased uptake

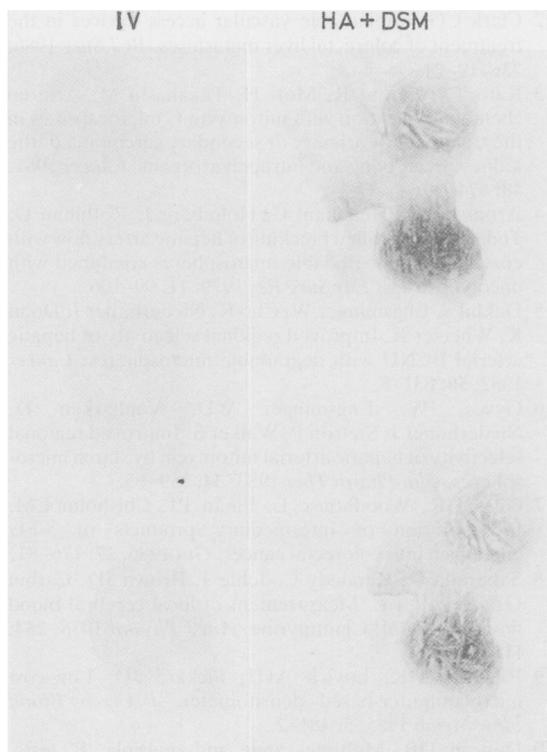


Fig. 5 Autoradiograms of sections containing liver tumours after iv infusion of  $^{14}\text{C}$ -5-FU alone and into the hepatic artery with DSM (HA). Tumours can be identified by the areas of increased density which is more intense in three tumours after hepatic arterial 5-FU with DSM compared with iv infusion.

of 5-FU after HAI with DSM compared with IVI (Fig. 5). The portal vein injections with or without DSM resulted in only a minimal increase in uptake which was not significant. This is partly a reflection of the very small component of overall portal venous flow that supplies the tumours and this would result in a very small fraction of the dose of 5-FU passing through the tumours during the first pass.

### Discussion

Spontaneous liver metastases in the rat from azoxymethane induced primary colorectal carcinoma has been described by Jenkins *et al.*<sup>11</sup> Liver metastases developed in only 47 of 180 rats after 40 weeks. The long time interval and relatively low yield is a disadvantage for any experimental design. The Walker 256 tumour, when injected as a suspension into the portal vein or directly implanted into the liver consistently resulted in liver tumours within two weeks.<sup>12,13</sup> The tumour used in these experiments

(MC-28) consistently resulted in multiple liver tumours that were suitable for study at 16 days. This is very convenient for experimental design. The rapid growth of the tumours and general deterioration of the rats after 20 days, however, limits certain aspects related to the evaluation of therapeutic modalities for liver metastases, such as the effect on survival or tumour necrosis after hepatic artery ligation.

The blood flow studies reflect findings previously made experimentally<sup>14</sup> and in man with colorectal liver metastases.<sup>15</sup> It has not been stressed previously, however, that a T/L ratio derived after an intraportal or intra-arterial injection of microspheres is simply a measurement of the vascular distribution to the tumours relative to normal liver. The method does not take into account the widely different perfusion rates in the respective vessels. Hepatic arterial perfusion in the rat can be quantified directly and total portal venous flow indirectly, by using the microsphere 'reference' sample technique. One study showed that total liver blood flow was 1.68 ml/g/min and hepatic arterial flow 0.29 ml/g/min in the rat.<sup>16</sup> The technique cannot quantify regional portal flow, however. Taking the wide discrepancy in portal venous flow and hepatic arterial flow into account, a low T/L ratio resulting from intraportal injections of microspheres does not imply that portal venous blood accounts for an equally low fraction of total tumour blood flow as suggested by Ackerman *et al.*<sup>12</sup> and Blanchard *et al.*<sup>17</sup>

Quantitative autoradiography has been found to be an extremely accurate method of measuring cerebral blood flow because of the high diffusibility of  $^{14}\text{C}$ -iodoantipyrine.<sup>9</sup> It has not been used previously for the measurement of liver blood flow. It is particularly useful for studying regional blood flow within tumours for which the microsphere method is not. A limitation of the technique occurs because the pre-portal organs would have extracted a certain amount of  $^{14}\text{C}$ -iodoantipyrine before its arrival in the liver. In this light it was felt that the actual blood flow could not be calculated accurately and that only a T/L should be derived. The uptake of the tracer is, however, directly related to tissue perfusion and not simply a measure of arterial or portal distribution.

The results of the blood flow studies using  $^{14}\text{C}$ -iodoantipyrine support the evidence that tumours are capable of deriving a significant fraction of the portal venous flow after HAL. Taylor *et al.*<sup>15</sup> found that, although colorectal liver metastases have a predominant arterial blood supply, they obtained portal venous blood after HAL using the xenon-133 clearance technique. It has been shown more recently after the injection of different coloured 'microfil' into the hepatic artery and portal vein that metastases in necropsy specimens of livers almost invariably had a

dual blood supply.<sup>18</sup> Earlier studies were unaware of the potential importance of the portal vein for the blood supply of liver tumours.<sup>19,20</sup> Relative tumour perfusion is markedly less than normal liver after PVL. It can only be postulated that the increased hepatic arterial perfusion that occurs in such circumstances<sup>21</sup> goes preferentially to the normal liver.

The results of 5-FU uptake by tumours should be considered in the light of the results from the microsphere and <sup>14</sup>C-iodoantipyrine control group blood flow studies. It is known that DSM increases the hepatic uptake of 5-FU in normal livers after intra-arterial administration in the rat.<sup>22</sup> Pharmacokinetic studies in man have shown that the systemic concentrations of cytotoxic drugs are considerably reduced when given into the hepatic artery with DSM.<sup>5,6</sup> These studies show that DSM significantly increases the uptake of 5-FU by tumours after arterial administration in spite of the hepatic artery being extremely small and some loss of the infusion by retrograde flow into the splanchnic vessels.

The role of DSM given into the portal vein has not been previously described presumably because of the fear of precipitating portal vein thrombosis with a longterm catheter and the risks of recurrent temporary blood flow stasis. Degradable starch microspheres did not enhance the uptake of 5-FU after intraportal injection as the hepatic artery had not been ligated and in view of the much greater overall portal venous flow, a much larger intraportal dose would be required to achieve the same concentration in the tumours as that following the intra-arterial route. It appears that although some tumours have a significant portal blood supply overall, the greatest uptake of 5-FU is achieved by selective hepatic arterial infusion with DSM.

In conclusion, liver metastases were reliably produced in the rat after the injection of sarcoma cells into the mesenteric vein. The tumours are similar to colorectal liver metastases in man by their relative avascularity, a predominant arterial blood supply but a potentially important portal venous supply. Delivery of chemotherapy by the hepatic artery results in increased uptake by the tumours when mixed with DSM.

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