LIVER

Location and function of intrahepatic shunts in anaesthetised rats

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Background: In the present study we determined the proportion of shunt flow due to patent intrahepatic portal systemic shunts in the normal rat liver and its relationship with microsphere induced portal hypertension.

Methods: Systemic and hepatic haemodynamics were measured continuously before, during, and after intraportal injection of 15 µm diameter microspheres in anaesthetised male Wistar rats. Functional hepatic blood flow and intrahepatic shunt flow were determined by the use of constant intraportal infusion of sorbitol and simultaneous measurements in the portal vein, hepatic vein, and carotid artery. The percentage of large shunts of diameter >15 µm were estimated by intraportal injection of ^{51}Cr labelled 15 µm diameter microspheres.

Results: Hepatic sorbitol uptake was 97.9 (0.5)% in normal control rats, with functional hepatic blood flow equalling total hepatic blood flow (2.52 (0.23) ml/min/100 g body weight). Microsphere injection decreased sorbitol uptake to 12.8 (4.3)% and further to 4.1 (0.7)% when followed by hepatic arterial ligation. In the latter two groups, intrahepatic shunt flow (1.46 (0.15) and 1.16 (0.19) ml/min/100 g body weight, respectively) was not significantly different from portal venous flow (1.36 (0.20) and 1.20 (0.20) ml/min/100 g body weight, respectively). Portal venous flow remained at 70% of basal values and portal venous pressure only increased by 50% from baseline. ^{51}Cr labelled microsphere shunt fraction through large shunts (>15 µm) was less than 1.0%.

Conclusion: The site of confluence between the hepatic artery and portal vein is in zone II. Intrahepatic shunts originate in presinusoidal regions in zone I in the normal liver and, when activated by intraportal injection of microspheres, divert 70% of the total portal blood flow away from zone III and thereby reduce acute increases in portal venous pressure.

Portal blood, the aggregate of splanchnic flow from the gut, spleen, pancreas, and gall bladder, accounts for approximately 75% of total hepatic blood flow.[1] The portal vein divides into preterminal and terminal portal venules within the liver[2] and are 40–80 µm and 15–35 µm in diameter, respectively, in the rat.[3] It was assumed that intrahepatic portal systemic shunting was negligible in the normal liver[4] but common in cirrhosis.[5] In well developed cirrhosis, intrahepatic shunts are small, <15 µm in diameter, in rats,[6] but larger in dogs[4] and humans.[7] We have demonstrated the existence of intrahepatic shunts, with diameters of up to 80–90 µm, in the normal rat liver using conventional physiological indices[8 9] and have confirmed their existence in the dual perfused normal rat liver using glucose uptake measurements where intrahepatic portal blockade by microspheres significantly increased intrahepatic shunting, as indicated by the reduced portal venous uptake of glucose but retained bromosulphthalein (BSP) uptake.[10–13]

Further in vivo studies of intrahepatic shunting have been hindered by the lack of a reliable technique to measure functional hepatic blood flow and intrahepatic shunt flow. Radioactive microsphere techniques measure the shunt fraction by recording pulmonary recovery of microspheres usually injected intraportally.[14 15] However, the shunt fraction measured by radiolabelled microspheres is limited by the 15 µm diameter of microspheres used, and by definition, blood flow through small shunts (<15 µm) is excluded. Most clearance methods currently available for human studies, including sorbitol clearance, require long infusion times, numerous large volumes of blood,[16–20] and are thus unsuitable for use in small animals such as the rat.

Long infusion times and large boluses were avoided in the present study by giving sorbitol intraportally in the rat, instead of systemically, as in human studies. Sorbitol was selected because it is safe, easy to measure, and has a high extraction ratio in the normal liver without interference from glucose.[16–19] Thus the aims of the present study were to determine: (i) the proportion (shunt fraction) of intrahepatic shunt flow in the normal rat liver following intrahepatic portal occlusion by microspheres; (ii) the anatomical origin of these shunts in the normal liver; and (iii) the site of confluence between the hepatic artery and portal vein.

MATERIALS AND METHODS

Surgical procedures

Male Wistar rats (300–350 g) allowed food and water ad libitum, were anaesthetised with fentanyl/fluanisone (0.3 ml/kg subcutaneously) and midazolam (0.3 ml/kg subcutaneously). Following heparinisation (30 U/100 g intravenously), the left femoral artery was cannulated for measurement of heart rate and mean arterial pressure. The left carotid artery and right jugular vein were cannulated for blood sampling and volume replacement, respectively. The abdomen was opened via a midline incision and three distal ileocolic veins were cannulated (2 FG catheters, 0.7 mm OD). One ileocolic catheter was introduced 1 cm for microsphere injection and sorbitol infusion, and the remaining two were advanced to the main trunk of the portal vein for measurement of portal venous pressure and portal blood sampling, respectively. The portal vein and hepatic artery were then carefully separated

Abbreviations: BSP, bromosulphthalein; PVF, portal venous flow; HAF, hepatic arterial flow; THBF, total hepatic blood flow; FHBF, functional hepatic blood flow; IPSS, intrahepatic portal systemic shunting.

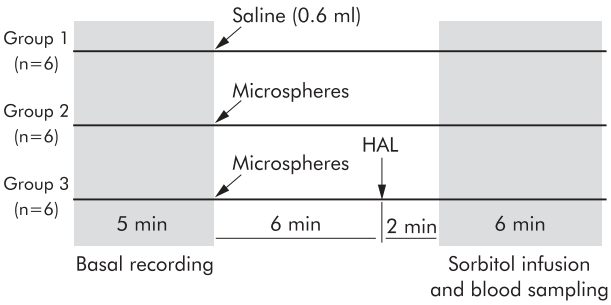

Figure 1 Experimental protocol used for the three groups which were given an intraportal injection of saline (group 1, n=6), 15 µm diameter microspheres (group 2, n=6), or microspheres plus hepatic arterial ligation (HAL) (group 3, n=6).

and an electromagnetic probe placed around each of them for flow measurements. A loose tie of 7-0 silk was placed around the hepatic artery for hepatic arterial ligation. Hepatic venous blood samples were obtained[21] using a catheter with a needle tip, which was carefully inserted through the parenchyma of the left liver lobe into the left hepatic vein, where it was advanced 5–10 mm to the confluence of the left and right hepatic veins. The position of this cannula was confirmed at post mortem. Pressures were measured on P23XL (Viggo Spectramed Inc, Carolina, Medical Inc., South Carolina, USA) pressure transducers and blood flow on electromagnetic flowmeters (SP 2202, Spectramed Inc.), which were both connected to a Polygraph (P37) recorder (Grass Instruments Inc., Quincey, Massachusetts, USA) for recording of permanent traces. Temperature was maintained at 37±0.5°C by a heating blanket and monitored by a rectal probe (MC 9200; Exacon, Roskilde, Denmark).

Experimental design

When stable basal values were obtained for at least five minutes, rats were given an intraportal injection of saline (group 1, n=6), 15 µm diameter microspheres (group 2, n=6), or microspheres plus hepatic arterial ligation (group 3, n=6) (fig 1). Hepatic arterial ligation was performed following injection of microspheres. This was conducted by tightening the 7-0 silk tie previously placed around the hepatic artery at least three minutes after stable values were obtained. Intraportal infusion of sorbitol (10 mmol/l, 0.2 ml/min) commenced two minutes after microsphere or saline injections or hepatic artery ligation. Two minutes after initiation of sorbitol infusion, blood samples were drawn simultaneously from the carotid, portal venous, and hepatic venous catheters. Blood (0.6 ml) was taken from each catheter over a six minute period and transferred into test tubes for sorbitol measurement. To compensate for the difference between the volume infused (0.2 ml/min) and the blood withdrawn (0.3 ml/min), fresh heparinised rat blood, prepared just before the experiments, was infused (0.1 ml/min) during blood sampling via the jugular catheter using a micropump (2120 Varioperpex II Pump; LKB Bromma, Sweden). Hepatic arterial flow was measured discontinuously before microsphere injection and after blood sampling to avoid electrical interference between the portal and hepatic arterial flow probes. Continuous traces of hepatic arterial flow were obtained from four additional rats for each of the groups above. On termination of the experiments, all rats were killed with an overdose of anaesthetic. Livers were excised for histology after perfusion through the portal vein with a 10% solution of formaldehyde in phosphate buffered saline at a physiological flow rate of 6–8 ml/min.[9]

Unfasted rats were used in this study and this may have resulted in blood glucose interference with sorbitol uptake. Therefore, in another six rats (group 4), hepatic sorbitol uptake was measured during continuous intravenous infusion of glucose 1 M at 0.2 ml/min, the rate estimated to increase basal blood glucose from 5 to 20 mM. Physiological sorbitol concentrations in the portal vein, hepatic vein, and carotid artery were also measured in an additional group of six rats (group 5).

To examine intrahepatic distribution of the injected microspheres and the proportion of shunting through large intrahepatic portal systemic shunts (>15 µm in diameter), ^{51}Cr labelled microspheres were injected via a portal venous catheter to a further three groups of rats—that is, given alone (group 6, n=6), with (group 7, n=6), or after (group 8, n=6) unlabelled microsphere injection (see below). Ten minutes after injection of ^{51}Cr labelled microspheres, animals were killed with an overdose of anaesthetic. The lobes of each liver

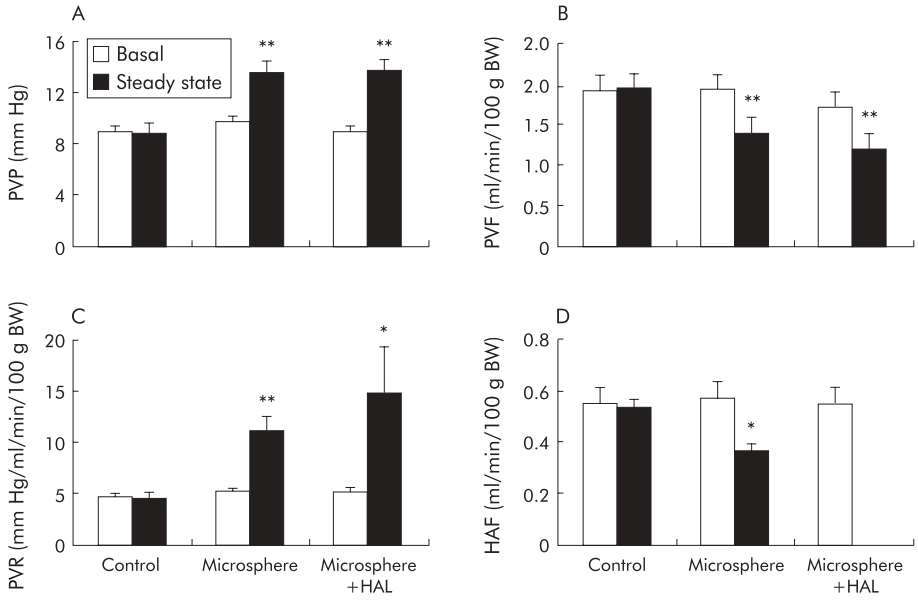

Figure 2 Changes in (A) portal venous pressure (PVP), (B) portal venous flow (PVF), (C) portal venous resistance (PVR), and (D) hepatic arterial flow (HAF) in rats that received saline (control), microsphere injection (microsphere), and microsphere injection plus hepatic arterial ligation (microsphere+HAL). Basal and steady state values are compared. *p<0.05, **p<0.01, Student's paired *t* test, steady state versus basal.

Table 1 Hepatic sorbitol uptake and sorbitol concentrations in the portal vein, hepatic vein, and carotid artery

Group	Treatment	Hepatic sorbitol uptake (%)	Sorbitol concentration (mmol/l)		
			Portal vein	Hepatic vein	Carotid artery
1	Control	97.9 (0.5)	0.432 (0.088)	0.006 (0.001)	0.004 (0.001)
2	Microsphere	12.8 (4.3)**	0.388 (0.080)	0.284 (0.051)**	0.128 (0.012)**
3	Microsphere+HAL	4.1 (0.7)**††	0.533 (0.070)	0.511 (0.070)**††	
4	Glucose	97.5 (0.6)	0.562 (0.079)	0.008 (0.002)	0.016 (0.006)
5	Physiological		0.014 (0.004)‡‡		

Values are mean (SEM).
HAL, hepatic arterial ligation.
**p<0.01 versus control and glucose; ††p<0.01 versus microsphere; ‡‡p<0.01 versus other groups (Student's unpaired *t* test).

and lung were numbered, weighed, and cut into small pieces. The radioactivity of each organ was determined in a gamma scintillation counter (Cobra II Auto Gamma; Canberra Packard, Pangbourne, Berks, UK).

Injection of microspheres

Microspheres were injected as described in our previous studies.[8 9] Briefly, counted latex microspheres or ^{51}Cr labelled microspheres (Coulter Electronics Ltd, Harpenden, Hertfordshire, UK), 15 (0.3) µm in diameter, were dispersed using an ultrasonic bath and then mixed using a vortex shaker for 60 seconds. In groups 2 and 3, 0.6 ml of unlabelled microspheres (1.7×10^7) were injected via the portal venous catheter over 10–15 seconds. This number of microspheres was selected for the present study because this volume of microspheres, when delivered intraportally, had previously been shown to be sufficient to achieve intrahepatic portal occlusion, as indicated by maximal increases in portal venous pressure.[9]

Similarly, in group 6, a 0.2 ml suspension containing approximately 3×10^4 ^{51}Cr labelled microspheres was injected into the portal venous catheter over 15 seconds. This technique has been used widely in the rat for estimation of portal systemic shunting[14 15] and intrahepatic distribution of portal blood flow.[22 23] In group 7, labelled (3×10^4) and unlabelled (1.7×10^7) microspheres were vortexed before injection into the portal vein. In group 8, labelled microspheres (3×10^4) were given five minutes after injection of unlabelled microspheres because pressures and flows stabilised at this time point, as indicated by the results of groups 2 and 3.

Infusion of sorbitol

D-Sorbitol (10 mM) (Sigma, St Louis, Missouri, USA) in saline was used in this study and always freshly prepared with 1% bovine serum albumin (Sigma). Sorbitol was infused using a peristaltic roller micropump (2120 Varioperpex II Pump; LKB Bromma). The pump infusion rate was kept constant at 0.2 ml/min in each rat. In our preliminary study, when different concentrations of sorbitol (5, 10, 15, and 20 mM) were infused intraportally at this rate, portal sorbitol was easily measured (0.2–2.0 mM) and hepatic sorbitol uptake was nearly complete (97%, 95%, 96%, and 95%, respectively).

Sorbitol assay

Blood samples were centrifuged at 2500 *g* for 15 minutes and plasma samples stored at –20°C until analysis. Sorbitol concentrations were measured by an enzymatic-spectrophotometric method.[16 20] Briefly, 0.15 ml of 10% cold perchloric acid were added to 0.3 ml of plasma and this was placed at 4°C for 24 hours for plasma deproteinisation. Following centrifugation, 0.2 ml of supernatant were neutralised with 0.05 ml 2 M K_2CO_3. After 15 minutes, centrifugation was performed again and 1 ml of buffer (49.6 mg/ml $Na_4P_2O_7$, pH 9.5) with 0.28 mg/ml sorbitol dehydrogenase and 1.8 mg/ml nicotinamide adenosine dinucleotide were added to 0.2 ml of supernatant. This was left at 20°C for 60 minutes before absorption was read at 340 nm on a spectrophotometer (Cecil 20/20; Cecil Instruments Ltd, Cambridge, UK), with buffer as a blank. All samples were assayed as one set, and the standard containing 0, 0.125, 0.25, 0.5 1.0, and 2.0 mM sorbitol was measured at the same time.

Calculations and statistics

Portal venous flow (PVF) and hepatic arterial flow (HAF) were measured as ml/min/100 g body weight. Total hepatic blood flow (THBF) equalled PVF+HAF. Portal venous resistance was calculated as mm Hg/portal blood flow. Assuming that the sorbitol concentrations in the carotid artery and hepatic artery were equal, hepatic sorbitol uptake was calculated using the following formula:

$$\text{Hepatic sorbitol uptake (\%)} = ((PVF\times S_{PV}+HAF\times S_{CA})-(PVF+HAF)\times S_{HV}/PVF\times S_{PV}+HAF\times S_{CA})\times 100$$

where S_{PV}, S_{CA}, and S_{HV} refer to sorbitol concentrations in the portal vein, carotid artery, and hepatic vein, respectively. Functional hepatic blood flow (FHBF) was calculated as THBF×hepatic sorbitol uptake and intrahepatic shunt flow calculated as THBF–FHBF.

Intrahepatic distribution of microspheres was expressed as lobe radioactivity (cpm)/total hepatic radioactivity (cpm). Intrahepatic portal systemic shunting (IPSS) was calculated from injection of ^{51}Cr labelled microspheres as follows[15]:

$$\text{IPSS (\%)} = \text{Lung radioactivity (cpm)}\times 100/\text{liver radioactivity (cpm)}+\text{lung radioactivity (cpm)}$$

Results are expressed as mean (SEM). Statistical comparisons within and between groups were performed using Student's paired and unpaired *t* tests, respectively. For repeated measurements at different time courses, multivariate analysis of variance (ANOVA) was applied and, when a difference was found, followed by Student's paired *t* test and Bonferroni adjustment. Results were considered statistically significant at $p<0.05$.

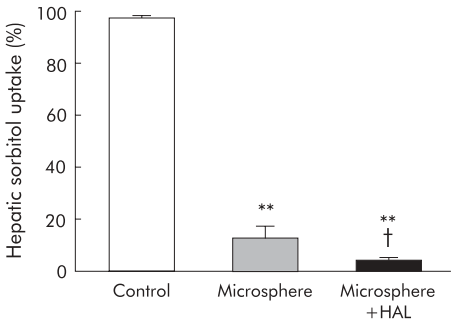

Figure 3 Hepatic sorbitol uptake in rats receiving saline (control), microsphere injection, and microsphere injection plus hepatic arterial ligation (HAL). **p<0.01 versus control; †p<0.05 versus microspheres, Student's unpaired *t* test.

Table 2 Hepatic blood flows

Group	Treatment	PVF	HAF	THBF	FHBF	IHSF
1	Control	1.95 (0.18)	0.57 (0.05)	2.52 (0.23)	2.51 (0.20)	0.06 (0.02)‡‡
2	Microsphere	1.36 (0.20)*	0.36 (0.04)*	1.72 (0.23)*	0.25 (0.15)**	1.46 (0.15)**‡‡
3	Microsphere + HAL	1.20 (0.20)*		1.20 (0.20)**†	0.05 (0.01)**	1.16 (0.19)**‡‡

Values are mean (SEM) (ml/min/100 g body weight).
PVF, portal venous flow; HAF, hepatic arterial flow; THBF, total hepatic blood flow; FHBF, functional hepatic blood flow; IHSF, intrahepatic shunt flow.
*p<0.05, **p<0.01 versus control; †p<0.05 versus microsphere (Student's unpaired *t* test).
‡‡p<0.01 versus FHBF (Student's paired *t* test).

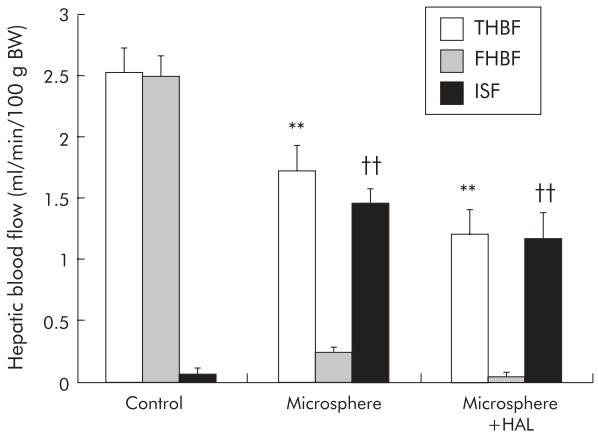

Figure 4 Comparison of total hepatic blood flow (THBF), functional hepatic blood flow (FHBF), and intrahepatic shunt flow (ISF) in saline (control), microsphere injection, and microsphere injection plus hepatic arterial ligated (HAL) rats. **p<0.01 versus controls, Student's unpaired *t* test; ††p<0.01 versus functional hepatic blood flow, Student's paired *t* test.

RESULTS

Haemodynamics

There were no significant differences in basal values of hepatic haemodynamics between the different study groups (p>0.05). Following microsphere injection, portal venous pressure immediately increased by 115% in group 2 and group 3, from basal values of 9.7 (0.4) mm Hg and 8.8 (0.4) mm Hg to peak values of 20.8 (1.6) mm Hg and 19.3 (0.9) mm Hg, respectively (p<0.01). Peak pressures declined within eight minutes to steady state values of 13.6 (0.5) mm Hg and 13.7 (0.8) mm Hg in group 2 and group 3, respectively, and remained 50% higher than basal values (p<0.01) (fig 2A).

In groups 2 and 3, microsphere injection immediately decreased portal venous flow by 50%, from 1.84 (0.17) ml/min/100 g body weight and 1.74 (0.11) ml/min/100 g body weight to 0.93 (0.19) ml/min/100 g body weight and 0.98 (0.19) ml/min/100 g body weight (p<0.01, as above). Portal venous flow partially recovered within five minutes to steady values of 1.36 (0.20) ml/min/100 g body weight and 1.20 (0.20) ml/min/100 g body weight, approximating to 70% of basal values (p<0.01) (fig 2B). Hepatic arterial flow, measured continuously in the additional group of rats, showed a sustained reduction (40%) from basal values after microsphere injection. Similarly, hepatic arterial flow decreased by 37% following microsphere injection in group 2, from 0.57 (0.05) ml/min/100 g body weight to 0.36 (0.04) ml/min/100 g body weight (p<0.05) (fig 2D). In group 3, hepatic arterial ligation (fig 2D) did not generate significant changes in portal venous pressure and portal venous flow.

Consistent with the peak changes in portal venous pressure and flow, the calculated peak portal venous resistance initially increased by 400% in group 2 and by 380% in group 3. This remained at 110% and 160% higher than basal values at steady state (p<0.05) (fig 2C).

Microsphere injection in groups 2 and 3 induced an initial reduction in mean arterial pressure by 42% and 36%, respectively (p<0.01), followed by a small recovery which remained at 28% and 25% less than basal values (p<0.05). Heart rate (420–440 beats/min) did not show any significant changes during the experiments. Hepatic arterial ligation did not significantly change mean systemic arterial pressure or heart rate in group 3.

Sorbitol data

Haemodynamic parameters did not show any significant alterations during sorbitol infusion and blood sampling. In normal unfasted rats, physiological portal sorbitol concentrations were very low (0.014 (0.004) mmol/l) and undetectable in the hepatic vein and carotid artery (table 1). Simultaneous infusion of glucose did not affect sorbitol measurements compared with data from the control group.

Hepatic sorbitol uptake was nearly complete in control rats (97.9 (0.5)%) (table 1, fig 3) and rats which received simultaneous glucose infusion (97.5 (0.6)%). Microsphere injection decreased hepatic sorbitol uptake to 12.8 (4.3)%, and was further decreased to 4.1 (0.7)% (p<0.05) by hepatic arterial ligation (table 1, fig 3).

In control rats, 77% of the blood flow to the liver was via the portal vein, with the remaining 23% supplied via the hepatic artery (table 2). The near complete sorbitol uptake by the normal liver suggested full and functional contact of the portal

Table 3 Comparison of percentage lobe weight and percentage lobe radioactivity (cpm) following intraportal injection of ^{51}Cr labelled microspheres given alone, mixed, or after administration of unlabeled microspheres

Lobe No	Lobe weight/total liver weight (%)			Lobe cpm/total liver cpm (%)		
	Alone (group 6)	Mixed (group 7)	After (group 8)	Alone (group 6)	Mixed (group 7)	After (group 8)
1	9.2 (0.6)	8.3 (0.4)	8.9 (0.5)	16.9 (1.7)**	13.7 (4.4)*	13.5 (1.8)**
2	13.7 (0.3)	14.6 (0.4)	14.2 (0.7)	16.1 (1.7)	17.9 (4.0)	17.4 (1.8)
3	28.2 (0.5)	27.9 (0.4)	26.9 (0.6)	25.6 (2.7)	27.5 (1.9)	26.7 (2.2)
4	9.8 (0.4)	10.2 (0.5)	9.8 (0.5)	8.3 (1.5)	9.2 (1.7)	9.8 (2.0)
5	29.6 (0.5)	29.7 (0.5)	30.5 (0.7)	22.0 (2.6)	21.0 (2.1)	22.4 (1.3)
6	9.5 (0.4)	9.3 (0.4)	9.7 (0.1)	11.1 (0.9)	10.8 (3.5)	9.8 (1.6)

Values are mean (SEM)
*p<0.05, **p<0.01 versus lobe weight/total liver weight (Student's paired *t* test).

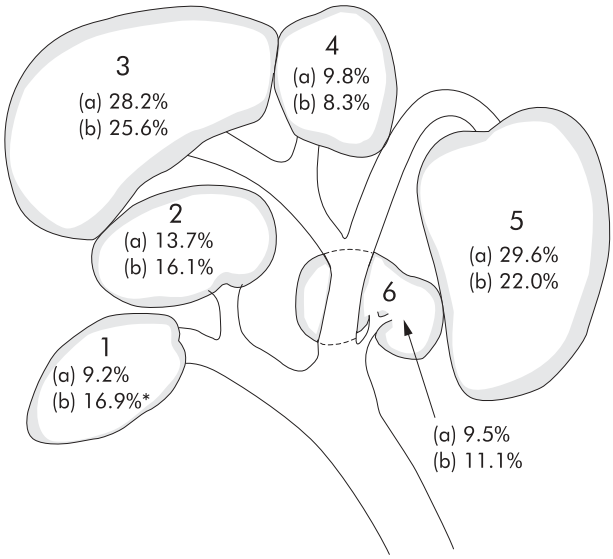

Figure 5 Illustration of the six liver lobes (numbered) with: (a) individual weight as a percentage of total liver weight and (b) radioactivity in each lobe as a percentage of the total in the liver of group 6, after intraportal injection of ^{51}Cr labelled microspheres. *p<0.05 versus percentage lobe weight, Student's paired *t* test.

Table 4 Intrahepatic portal systemic shunting (IPSS) measured by ^{51}Cr labelled microspheres

Group	Treatment	IPSS (%)	Range (%)
6	Alone	0.06 (0.01)	0.03–0.10
7	Mixed	0.22 (0.09)	0.02–0.51
8	After	0.63 (0.40)	0.04–2.10

blood with the hepatic sinusoids—that is, total hepatic blood flow equalled functional hepatic blood flow (table 2, fig 4). Following microsphere injection, total hepatic blood flow decreased by 30%, consistent with reductions in mean arterial pressure, functional hepatic blood flow was greatly reduced, and intrahepatic shunt flow increased (table 2, fig 4). This became more pronounced following hepatic arterial ligation (table 2, fig 4).

Labelled microsphere data

No significant differences in liver weight were found between the different groups after injection of ^{51}Cr labelled microspheres ($p>0.05$). Results from groups receiving labelled microspheres alone (group 6), mixed (group 7), or after (group 8) injection of unlabelled microspheres are compared in table 3. Intrahepatic distribution of injected microspheres was generally homogeneous in all of the three groups (table 3). However, significantly increased radioactivity of 13.5–16.9% was found in lobe 1 which represented 8.3–9.2% of the total liver weight (table 3). The six liver lobes, percentage lobe weight, and percentage lobe radioactivity (cpm) in group 6 is illustrated in fig 5. The intrahepatic shunt fraction through large shunts (>15 μm in diameter), measured by ^{51}Cr labelled microspheres, was always less than 1% in all three groups (table 4).

Morphology and histology

Following microsphere injections, livers became pale and, on the liver surface, chains of microsphere clusters were seen along the portal tracts (fig 6). This was also observed in cross sections of cut lobes at the end of the experiments. Histological examination of the sections of the liver showed complete occlusion of terminal portal branches by the injected microspheres, with some microspheres lodged in the first one third of the sinusoids (fig 6).

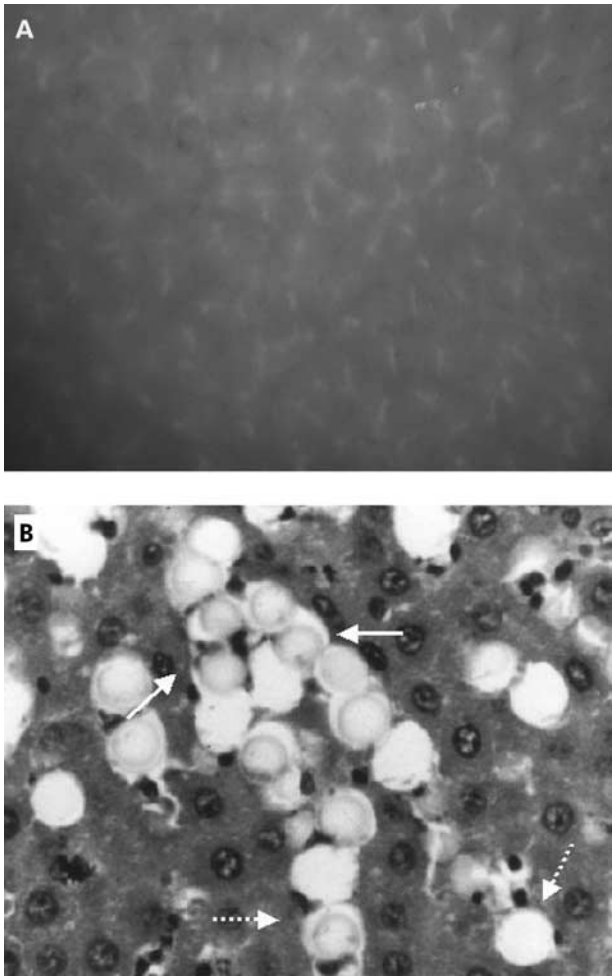

Figure 6 (A) Microspheres distributed along the preterminal and terminal portal branches, forming "tree-like" microsphere clusters (group 2, original magnification ×9). (B) Microspheres lodged in the terminal portal branches (solid arrows) and some lodged in the first one third of the sinusoids (broken arrows) (group 2, original magnification ×40).

DISCUSSION

The present study demonstrated presinusoidal obstruction and high resistance to portal blood flow following microsphere injection. Microspheres were found predominantly in preterminal and terminal portal venules while a smaller proportion were lodged in the sinusoids, to form chains of microspheres. Total portal venous occlusion induces a maximal increase in portal venous pressure of 40–60 mm Hg.[8 9] This represents more than a 700% increase in portal venous pressure. The discrepancy in pressure increases observed in the present study between microsphere induced and total portal venous occlusion was consistent with the existence and opening of intrahepatic portal systemic shunts in the normal rat liver.[8 9] Our previous studies clearly demonstrated that an acute rise in portal venous pressure is to be expected following any form of portal venous occlusion, be it cross clamping or injection of microspheres into the portal vein.[8] This is accompanied by a substantial decrease in systemic blood pressure. The rise in portal venous pressure is sustained if portal hepatic occlusion is total and appears to peak at an average pressure of 60 mm Hg.[9] However, if the occlusion is transient, the acute rise in

portal venous pressure subsides simultaneously with alleviation of the pressure. Whether this is achieved by total or partial removal of a portal venous cross clamp or by opening of the additional blood conduits (in this instance intrahepatic shunts) appears immaterial to the alleviation of pressure. However, it seems that it is irrelevant whether the rise in portal venous pressure is sustained or alleviated, in both instances a significant and substantial decrease in systemic blood pressure is sustained that suggests that either neural innervation or a chemical mediator (or both) may be involved. Moreover, the data from the present study can only suggest that the systemic response is hepatically driven. The only direct way to answer these interesting and very relevant questions is to conduct experiments where the hepatic circulation is isolated from the systemic circulation. These experiments were beyond the scope of the present study but were conducted and have been presented elsewhere. They have clearly demonstrated that the response is indeed elicited by the liver, that it is not due to innervation, and that a chemical mediator is likely to be involved.[24] The response was subsequently termed "the hepatocardiovascular response" and appears to be elicited only following intrahepatic stimulation, during pathophysiological conditions, for example.[24]

It was originally believed that the acute fall in systemic blood pressure following microsphere injection into the portal vein was due to mesenteric pooling.[9] However, several observations from our experiments have led us to believe otherwise. Firstly, if mesenteric pooling were responsible for the acute decreases in systemic blood pressure this should have decreased progressively with increasing numbers and sizes (diameters) of microspheres as more sinusoids become occluded in the liver, and this was not observed.[24] Secondly, experiments were conducted where the volume of blood that was potentially trapped in the mesenteric circulation was calculated and replaced. The calculated decrease in blood volume that resulted in the same reduction in systemic blood pressure was not reversed by blood volume replacement.[25] Thirdly, experiments where the mesenteric circulation was excluded from the circulation showed that the fall in systemic blood pressure still occurred following portal occlusion by microspheres.[24] All of these observations led to the conclusion that mesenteric pooling was an unlikely cause of the systemic blood pressure reductions and that possibly an intrahepatic stimulus was responsible.

Microspheres were injected into a sufficiently distal ileocolic vein instead of the main trunk of the portal vein[9] to ensure optimal mixing within the portal tract before entering the liver. This was supported by the labelled microsphere data that indicated homogeneous mixing within the liver. The slight increase in microsphere distribution in lobe 1 was probably due to distribution of portal blood within the liver due to a short, wide, and straight route to lobe 1 (fig 5). Intrahepatic distribution of portal blood flow in the normal liver remains a controversial issue[22 23 26 27] and our data were consistent with the observations of Blei and colleagues[22] and Stuart and Wheatley[23] using the same technique.

Most of the advantages of using sorbitol as a test substance for assessment of functional hepatic blood flow in humans were also demonstrated in the present study in the rat. Sorbitol was normally undetectable in the hepatic vein and carotid artery and negligible in the portal vein. There was no interference with sorbitol measurement by glucose administration. The near complete sorbitol uptake in the normal rat liver indicated that the portal blood flow had full and functional contact with hepatocytes, and therefore under normal conditions functional hepatic blood flow equalled total hepatic blood flow.[16 19]

The question of the site of confluence of the hepatic arterial supply with the portal venous supply remains controversial.[28–30] Hepatic sorbitol uptake decreased to 13% following microsphere injection and this can be almost entirely attributed to hepatic arterial blood perfusing the sinusoids. Where microsphere injections were followed by hepatic arterial ligation, to exclude hepatic arterial sorbitol uptake, hepatic sorbitol uptake decreased further to 4.3%, resulting in a sorbitol concentration in the hepatic vein approaching that in the portal vein. This result indicated that 95.7% of portal flow bypassed the sinusoidal regions responsible for sorbitol uptake and was consistent with the intrasinusoidal confluence of portal and hepatic arterial flow. Our data demonstrate that sorbitol uptake via the hepatic artery is not affected by presinusoidal occlusion. This indicates that the main site of confluence of the hepatic artery with the portal vein is intrasinusoidal, in zone II, thus substantiating earlier work.[13] If this

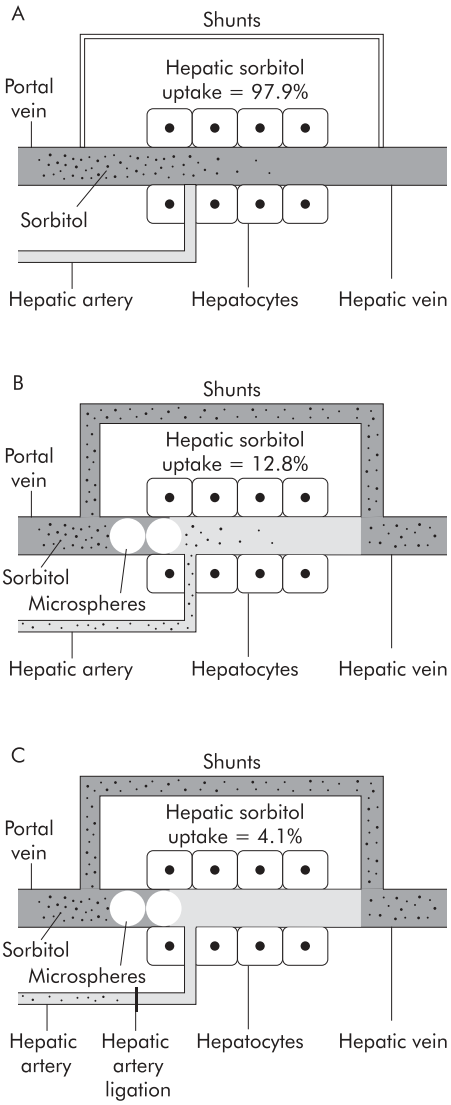

Figure 7 Illustration of the relationship between intrahepatic shunts, hepatic sorbitol uptake, and microsphere induced presinusoidal occlusion in the normal rat liver. (A) Under normal conditions, portal blood flows through the sinusoids with extremely low resistance. Intrahepatic shunts are normally closed and thus hepatic sorbitol uptake is complete. (B) Portal blood flowing to the sinusoids is stopped by microspheres injected intraportally. Intrahepatic shunts open as a result of the acute increase in portal pressure and permit up to 70% of the basal value of portal blood flow to bypass the sinusoids. No portal sorbitol uptake occurred, but uptake from the hepatic artery remained intact due to the intrasinusoidal confluence of the portal and hepatic arterial vasculatures (see discussion). The latter largely accounted for the remaining 12.8% sorbitol uptake. (C) When microsphere injection was followed by hepatic arterial ligation, hepatic sorbitol uptake further decreased to 4.1%.

were presinusoidal, microspheres should reduce hepatic arterial sorbitol uptake to the same extent as portal venous sorbitol uptake and this did not happen. Haemodynamic observations showed a limited reduction in hepatic arterial blood flow after microsphere injection, possibly secondary to the decreased mean arterial pressure. In the isolated perfused rat liver where hepatic arterial flow was constant, intraportal injection of microspheres did not produce any increase in hepatic arterial resistance.[10 11] This evidence indicates an intrasinusoidal site of confluence between the portal venous and hepatic arterial vasculatures. This does not preclude the possibility that a small proportion of hepatic arterioles could drain directly into the terminal portal venules,[29] but data from the present and earlier studies[9 10 13] are inconsistent with this as the main site of confluence between the two vasculatures. A proposed hypothesis regarding the relationship between intrahepatic shunts, hepatic sorbitol uptake, and presinusoidal occlusion by microspheres is illustrated in fig 7.

After presinusoidal occlusion, almost all of the portal venous flow is shunted, with limited changes in portal venous pressure, thus implying that these shunts are important in the decompression of portal tracts. In an attempt to determine flow via large shunts (>15 μm in diameter), ^{51}Cr labelled microspheres with a diameter of 15 μm were given alone, with or following injection of unlabelled microspheres. Less than 1% of the labelled microspheres appeared in the lung, presumably via shunts larger than 15 μm diameter. Therefore, the number of large shunts is very few in the normal rat liver. Moreover, less than 1% of the shunt fraction cannot account for the total portal blood flow passing through large shunts because sorbitol uptake would have been reduced only by a comparable amount of 1%. Portal sorbitol uptake was reduced by 96% after injection of 15 μm diameter microspheres and therefore this blood bypassed zone III, which is responsible for sorbitol (and presumably glucose) uptake[12 13 30] via shunts less than 15 μm diameter. Furthermore, intrahepatic portal systemic shunting of 15 μm radioactive microspheres in cirrhotic rats amounted to only 9.4%,[31] but the percentage shunting measured by clearance of lidocaine was 53.9%.[6] It would therefore appear that intrahepatic shunts are predominantly small (<15 μm) in normal and cirrhotic rat livers. Our data confirm those showing that intrahepatic shunts originate in zone I because the microspheres occlude at a presinusoidal location in the normal liver where changes in BSP uptake prior to microsphere injections remained insignificant.[12 13]

The hepatic artery was ligated only in group 3 to determine the percentage sorbitol uptake due to portal venous flow alone and also after microsphere injection. A reciprocal "buffer" response, shown as an increase in flow, would be expected by the portal vein to this manoeuvre and was not observed in the present study. However, our previous studies conducted in vitro clearly demonstrated that deliberately increasing portal venous flow by the same proportion of the hepatic arterial flow, which is some 70% higher than the anticipated buffer response in the portal vein, did not significantly alter glucose uptake from the portal vein.[13] More importantly, the residual 4.3% hepatic arterial uptake of sorbitol following microsphere injection indicated that 95.7% of portal flow now bypassed zone III. The present study demonstrated near complete sorbitol uptake in the portal vein when intrahepatic shunts are closed. Once the shunts are induced to open either via reductions in hepatic arterial blood flow[10] or by intraportal injections of microspheres, portal venous uptake of sorbitol decreases to extremely low values (13% in the present study) and 4.3% following hepatic arterial ligation. Therefore, the potential for increased uptake is high and would not be significantly reduced by increases in portal venous flow either by deliberate manipulation or through the buffer response. Moreover, if the intrahepatic shunts are open, any increases in portal venous flow are likely to be directed through the intrahepatic shunts where we now know that sorbitol and glucose uptake is minimal,[13] if anything at all.

Sorbitol was infused at 0.2 ml/min at a concentration of 10 mM, which is the normal blood glucose concentration. Portal venous flow rate was in the region of 2 ml/min/100 g body weight and a dilution of less than 0.1% with 10 mM sorbitol is hardly likely to exert any significant osmotic effects. Sorbitol is an isomer of D-glucose, differs in molecular weight by two hydrogen atoms, and shares the majority of the physical properties of glucose, including osmolarity.[32] Glucose or sorbitol do not exert any significant effects on portal flow due to their osmolarity at that concentration in the bloodstream, to the best of our knowledge. It is therefore unlikely that this concentration of sorbitol would have significantly affected portal blood flow.

It is possible that anaesthesia may affect the microcirculation. However, all of the rats used in the study were subjected to identical anaesthesia and the comparisons were drawn between control and test groups that were treated in an identical manner in terms of anaesthesia. In addition, some of the experiments conducted in the present study complied with data from earlier studies conducted in vitro,[10 13] where the effect of anaesthesia was insignificant. Finally, as all control and test groups were treated under identical conditions with regard to sorbitol concentrations, the effect, if there were any, would be common to all groups and therefore would not be responsible for the differences in results between groups. We therefore conclude that the effect of anaesthesia in the present study was also insignificant.

In summary, we have demonstrated in vivo that intrahepatic shunts may divert up to 70% of portal venous blood from zone III regions in the rat liver following intraportal microsphere injections. Intrahepatic shunts in the normal liver have predominantly diameters less than 15 μm and originate at zone I, presinusoidal regions. The site of confluence between the hepatic artery and portal vein occurs in the intrasinusoidal zone II. Intrahepatic shunts may have a functional and significant role in the modulation of portal venous pressure in acute portal hypertension.

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