

A new rat model of portal hypertension induced by intraportal injection of microspheres

LI Xiang-Nong¹, IS Benjamin² and B Alexander²

Subject headings portal vein; hypertension, portal; disease model, animal; latex microsphere

Abstract

AIM To produce a new rat model of portal hypertension by intraportal injection of microspheres. **METHODS** Measured aliquots of single or different-sized microspheres (15,40,80 μ m) were injected into the portal vein to block intrahepatic portal radicals. The resultant changes in arterial,portal,hepatic venous and splenic pulp pressures were monitored. The liver and lungs were excised for histological examination.

RESULTS Portal venous pressure was elevated from basal value of 0.89 - 1.02 kPa to a steady-state of 1.98 - 3.19 kPa following the sequential injections of single- or different-sized microspheres, with a markedly lowered mean arterial pressure. However, a small-dose injection of 80 μ m microspheres (1.8×10^5) produced a steady-state portal venous pressure of 2.53 ± 0.17 kPa, and all rats showed normal arterial pressures. In addition, numerous microspheres were found in the lungs in all experimental groups. **CONCLUSION** Portal hypertension can be reproduced in rats by intraportal injection of microspheres at a small dose of 80 μ m (1.8×10^5). Intrahepatic portal-systemic shunts probably exist in the normal rat liver.

INTRODUCTION

Portal hypertension is associated with gross haemodynamic disturbances in portal and systemic circulations. Animal models are still important for research into portal hypertension. One of the most popular models used is the partial portal venous ligation model in the rat^[1], but this can only achieve extrahepatic portal venous occlusion and is not representative of intrahepatic portal hypertension as seen clinically. Carbon tetrachloride induced models of cirrhosis closely resemble the major features of the human disease^[2], but take a long time to develop and are associated with a high mortality and a wide heterogeneity in the stage and development of cirrhosis^[3,4]. In the present study, a new rat model of portal hypertension was successfully induced by intraportal injection of microspheres.

MATERIALS AND METHODS

Animals

Thirty-eight Sprague-Dawley rats weighing 250 g - 350 g were randomly divided into six groups. Groups 1 ($n = 6$) and 2 ($n = 6$) received sequential injections of single-sized microspheres of 15 and 80 μ m diameters respectively. Groups 3 ($n = 6$) and 4 ($n = 6$) were given sequential injections of different-sized microspheres in order of size 15, 40 and 80 μ m and 80, 40 and 15 μ m, respectively. According to the results of the four groups above, two bolus injections of 80 μ m microspheres were selected as the suitable dose for the induction of portal hypertension in the model and these were given to Group 5 ($n = 8$) for further observation. Rats in Group 6 ($n = 6$) were injected with saline and served as controls.

Measurement of pressures

The animals were anaesthetised with fentanyl/fluanisone (0.3 ml/kg, subcutaneously) and midazolam (0.3 ml/kg, subcutaneously). Mean arterial pressure (MAP) was monitored using a catheter in the left carotid artery. The abdomen was opened via a midline incision and the portal vein was cannulated through an ileocolic vein for measurement of portal venous pressure (PVP) and injection of microspheres. The splenic pulp pressure (SPP) was measured through a 23G butterfly scalp needle. Wedged hepatic venous pressure (WHVP) was measured in Groups 1,2,5 and 6. All of the cannulae were connected to P23XL (Viggo Spectramed Inc.) pressure transducers, and

¹Department of Liver Surgery, Affiliated Hospital, Xuzhou Medical College, Xuzhou 221002, China

²Department of Surgery, King's College School of Medicine & Dentistry, London, UK.

Dr. LI Xiang-Nong, Associate Professor and Director of the Department of Liver Surgery, Affiliated Hospital, Xuzhou Medical College. Born on April 23, 1958. Graduated and obtained a bachelor degree from Xuzhou Medical College in 1982, and a master degree from Beijing Medical University in 1988. Worked as a visiting scholar in the Department of Surgery, King's College School & Dentistry in the UK from 1993-1994. Specialized in the study of portal hypertension and hepatic carcinoma; having 23 papers published. Tel. +86-516-5698950 ex 2003 or 2009

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Correspondence to Dr. LI Xiang-Nong, Department of Liver Surgery, Affiliated Hospital, Xuzhou Medical College, 50 Jiaheqian Street, Xuzhou 221002, China

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permanent recordings were made on a polygraph recorder (Grass Instruments Inc., USA). When steady basal pressures had been achieved for at least 5 minutes, injections of microspheres or saline started. Before injection, latex microspheres (Coulter Electronics Ltd., England) were agitated for 60 seconds. In each injection, microspheres were given iav the portal venous catheter in a volume of 0.2 ml and immediately followed by 0.2 ml saline injection to flush the catheter. Only when a steady PVP has been achieved for at least 5 minutes was the next injection given. In Group 5 there was no interval between the two injections. The numbers of microspheres used are shown in Table 1. Following completion of the injection, animals were observed for 10 - 30 minutes until the final steady PVP had been reached. Finally a vascular clamp was applied to the portal vein at the liver hilus and the pressures monitored. After this, the animals in Group 6 received partial portal venous ligation. At the end of the experiment, all rats were killed by opening the chest. The liver and lungs were taken and fixed in 10% formal-saline for histological examination.

Table 1 Intraportally injected aliquots of microspheres

Group	Sphere diameter (μm)	No of aliquots	No of spheres/aliquot	Total No of spheres
1	15	6	5.6×10^6	3.4×10^7
2	80	5	9.0×10^4	4.5×10^5
3	15,40,80	15μm×2	5.6×10^6	1.1×10^7
		40μm×2	2.4×10^5	4.8×10^5
		80μm×2	9.0×10^4	1.8×10^5
4	80,40,15	80μm×2	9.0×10^4	1.8×10^5
		40μm×2	2.4×10^5	4.8×10^5
		15μm×2	5.6×10^6	1.1×10^7
5	80	2	9.0×10^4	1.8×10^5

Note: Pressures showed no changes after the 4th and 5th injections in Group 2. Therefore the 6th injection was not given.

Statistics

Results were expressed as mean \pm standard error. Comparisons were made by means of *t* test. Results

were considered statistically significant at $P < 0.05$.

RESULTS

MAP decreased by approximately 45% following the first injection of microspheres in all experimental groups (kPa, 12.95 ± 1.66 vs 7.32 ± 0.68 , $P < 0.001$). MAP in Group 5 eventually returned to normal levels after approximately 40 minutes following the two injections of 80 μm microspheres. No change in MAP was observed in the control rats during saline injections and after partial portal venous ligation. However, portal venous occlusion in control rats produced a significant reduction in MAP (kPa, 12.86 ± 1.65 vs 6.88 ± 1.14 , $P < 0.001$), similar to that seen after microsphere injections in the experimental groups ($P > 0.05$).

PVP rose gradually following the microsphere injections and, in Group 1, synchronous increase in WHVP was found (kPa, 0.93 ± 0.13 vs 1.65 ± 0.24 , $P < 0.01$) (Figure 1). In Group 2, 80 μm diameter microspheres produced a large, rapid increase in PVP (Figure 2) with a significant reduction in WHVP (kPa, 0.89 ± 0.09 vs 0.47 ± 0.09 , $P < 0.01$). In Groups 3 and 4, a marked increase in PVP was only observed after injection of 80 μm diameter spheres (Figure 3). Four rats in Group 4 died within 2 - 17 minutes after the 6th injection of microspheres. In Group 5, two sequential bolus injections of 80 μm microspheres elicited an immediate reduction in WHVP (kPa, 0.92 ± 0.13 vs 0.47 ± 0.09 , $P < 0.01$) and an immediate increase in PVP to $2.53 \text{ kPa} \pm 0.17 \text{ kPa}$, which remained elevated during the observation period of 150 minutes. There were no significant changes in PVP in the control group after injection of an equivalent volume of saline aliquots. Results are compared in Table 2.

Portal venous occlusion with a clamp produced a further large, rapid rise in PVP (Figure 1) to $5.16 \text{ kPa} \pm 0.76 \text{ kPa}$, $4.87 \text{ kPa} \pm 0.79 \text{ kPa}$, $4.95 \text{ kPa} \pm 0.99 \text{ kPa}$, $5.55 \text{ kPa} \pm 0.89 \text{ kPa}$ and $5.75 \text{ kPa} \pm 0.32 \text{ kPa}$ in groups 1,2,3,5 and the control respectively. There was no significant difference between the values in these groups ($P > 0.05$). Partial portal venous ligation in control rats induced a significant increase in PVP to $2.75 \text{ kPa} \pm 0.07 \text{ kPa}$ and was similar to that in Group 5 ($P > 0.05$).

Table 2 Comparison of changes in portal venous pressure (PVP) and splenic pulp pressure (SPP) in different groups after microsphere injections

Group	PVP(kPa)				SPP(kPa)			
	Basal	Post-infusion	Basal-Post	% increase	Basal	Post-infusion	Basal-Post	% increase
1	0.98 ± 0.21	1.98 ± 0.48^a	1.00 ± 0.37^a	102.2 ± 35.6^a	1.17 ± 0.39	2.01 ± 0.51^a	0.84 ± 0.44^a	90.9 ± 41.8
2	0.89 ± 0.19	3.19 ± 0.29^a	2.30 ± 0.36^a	272.2 ± 78.0^a	1.44 ± 0.43	3.03 ± 0.33^a	1.60 ± 0.60^a	139.3 ± 51.7
3	1.02 ± 0.11	2.65 ± 0.49	1.64 ± 0.52	162.8 ± 60.1	1.24 ± 0.16	2.49 ± 0.45	1.24 ± 0.48	103.1 ± 48.5
4	0.98 ± 0.13	2.70 ± 0.13	1.72 ± 0.20	178.6 ± 41.0	1.16 ± 0.19	2.63 ± 0.13	1.49 ± 0.23	133.5 ± 39.2
5	1.04 ± 0.12	2.53 ± 0.17	1.45 ± 0.23^c	140.3 ± 32.0^c	1.44 ± 0.19	2.47 ± 0.19	1.04 ± 0.28^c	74.9 ± 26.6^a
6(control)	1.04 ± 0.07	1.02 ± 0.05			1.36 ± 0.20	1.37 ± 0.19		

^a $P < 0.05$, vs other groups; ^c $P < 0.05$, vs groups 3 and 4. Student's unpaired *t* test.

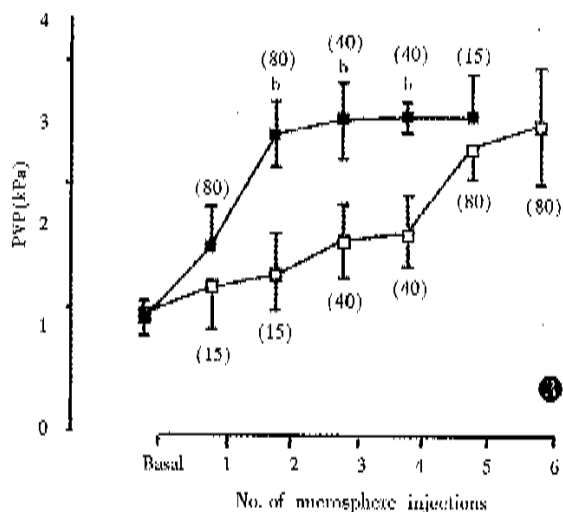
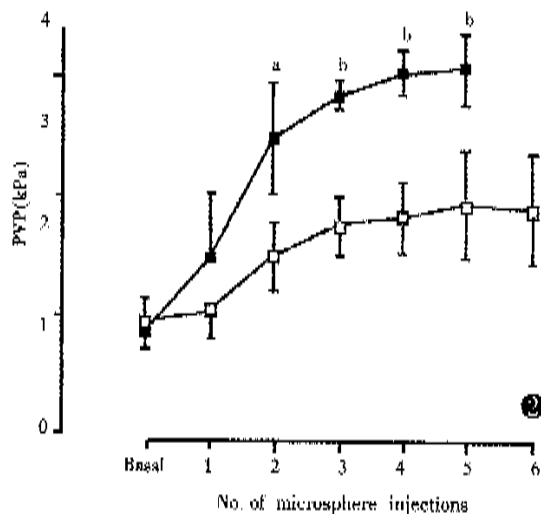
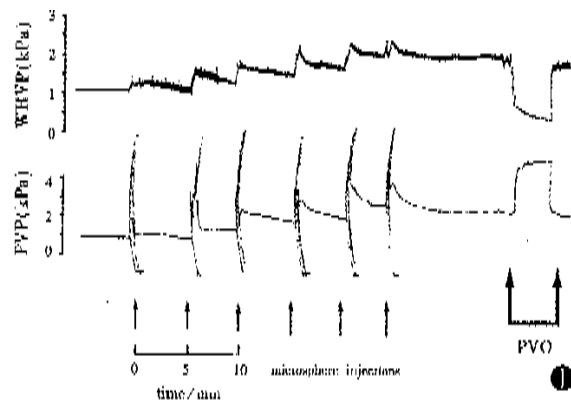


Figure 1 Portal venous pressure (PVP) and wedged hepatic venous pressure (WHVP) recordings obtained from a rat in Group 1 during intraportal microsphere injections and subsequent portal venous occlusion (PVO).

Figure 2 Changes in portal venous pressure (PVP) following intraportal microsphere injections in Group 1 (□) and Group 2 (■).

^a $P < 0.05$, ^b $P < 0.01$, Groups 2 vs Group 1.

Figure 3 Changes in portal venous pressure (PVP) following intraportal microsphere injections in Group 3 (□) and Group 4 (■). Four rats in Group 4 died after the 6th injection and therefore steady PVP recordings could not be obtained after this time. ^b $P < 0.01$, Group 4 vs Group 3.

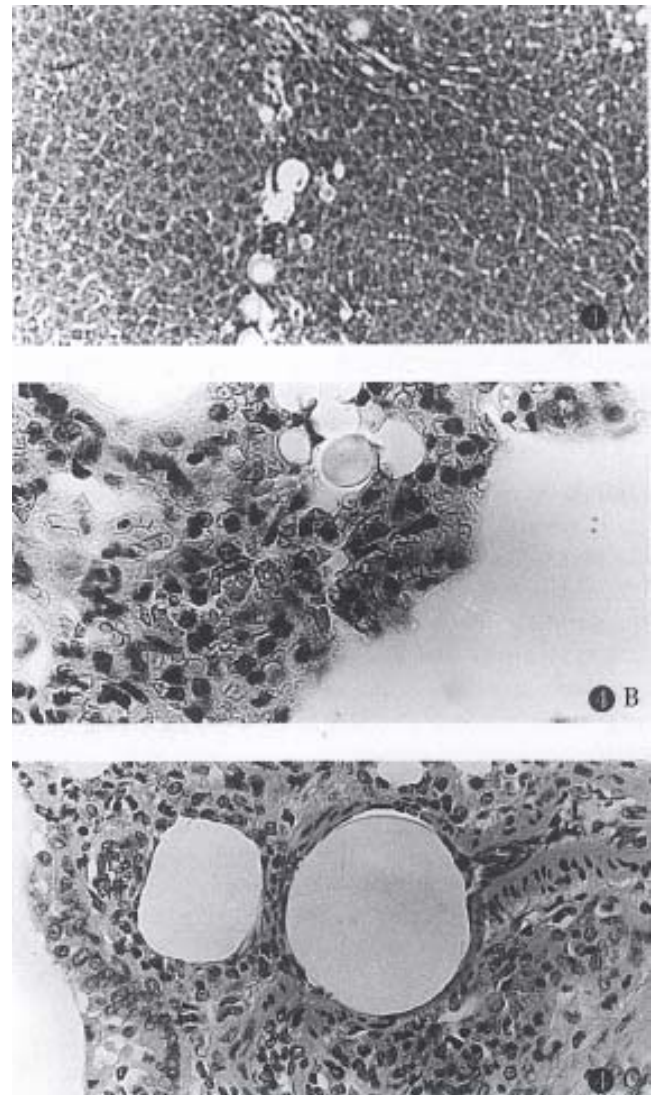


Figure 4 Histological sections showing (A) 15, 40 and 80 μ m spheres lodged in liver (Group 3, original magnification $\times 10$), (B) 15 μ m spheres lodged in lung (Group 1, original magnification $\times 40$) and (C) 80 μ m spheres lodged in lung (Group 2, original magnification $\times 16$).

Histological examinations showed that in Groups 1 and 2 almost all of the portal radicles identified in the fields were blocked by the injected microspheres, with 15 μ m spheres (Group 1) lodged in the terminal portal venules and 80 μ m spheres (Group 2) lodged in the large portal radicles. In Group 3, the large and small portal radicles were simultaneously blocked by the different sized spheres injected. However, in Group 4, portal venules were mainly obstructed by the 80 μ m spheres, while many 15 and 40 μ m spheres injected after the 80 μ m diameter spheres were trapped over the 80 μ m spheres. The results in Group 5 were similar to Group 2, but with fewer spheres than those observed in Group 2. Numerous microspheres were found in the lungs of all experimental groups (Figure 4).

DISCUSSION

Anatomically the intrahepatic portal tract branches into progressively smaller radicles until the sinusoids are reached. Therefore, intraportal injection of microspheres can block the intrahepatic portal radicles and lead to presinusoidal portal hypertension. The present study confirmed that the PVP was elevated to a steady state 100% - 270% above the basal value following microsphere injections. Obviously, the increase in PVP was related to the size and numbers of microspheres used: the 80 μm spheres caused a large, rapid rise in PVP compared to 15 μm spheres (Figure 2). As shown by the histology, the 80 μm spheres were trapped in large portal radicles and therefore smaller numbers would produce a marked PVP raising effect. The results of sequential injections of different-sized microspheres showed that the final PVP was dependent upon the effect of the largest spheres (Figure 3).

When intrahepatic portal radicles were blocked by the microspheres injected, the presinusoidal increase in resistance produced not only an elevated PVP, but also a markedly lowered MAP because of extensive mesenteric pooling of portalve nous blood. Therefore, both the PVP and MAP should be considered into the reproduction of this model. From the results of the first four groups, it was found that two injections (1.8×105) of 80 μm spheres created an augmentation in PVP with the recovery of MAP. Furthermore, all rats in Group 5 showed a normal MAP and high PVP of $2.53 \text{ kPa} \pm 0.17 \text{ kPa}$ which was comparable to the value of $2.75 \text{ kPa} \pm 0.07 \text{ kPa}$ in the control group after partial portal venous ligation, and to the value of $1.8 \text{ kPa} \pm 0.2 \text{ kPa}$ found in our cirrhotic model previously induced by carbon tetrachloride^[2]. These suggest that 1.8 ± 105 of 80 μm spheres is the suitable dose for induction of portal hypertension in the model.

Clinically, portal hypertension is mainly caused by different forms of chronic liver disease, and is characterized by an increased resistance in the liver and by the formation of portosystemic collaterals. Though in early studies the site of the increased resistance was thought to be postsinusoidal, recent works have proved that the presinusoidal resistance is also increased in cirrhotic liver and contributes to the development of portal hypertension^[5,6]. In particular, the presinusoidal block is the main cause of the idiopathic portal hypertension and the portal hypertension resulted from chronic biliary obstruction^[7,8]. Therefore, this model, in regard to the location of increased intrahepatic resistance, possesses the general features of clinical cases. Moreover, the fact that many microspheres appeared in the pulmonary vascular bed suggested the opening of portosystemic collaterals during the procedure, with a diameter of more than 15 μm - 80 μm .

Originally we believed that the presence of microspheres in the lung is only due to the opened extrahepatic collaterals, and this seems to be supported by the observation that the SPP was slightly lower than PVP. However, in this study although the PVP in groups 1 and 2 did not increase significantly after the 3rd injection, which implied that the intrahepatic portal radicles had been saturated by the microspheres injected, the final PVP achieved was substantially less than that obtained by portal vein occlusion. Consideration of the simultaneous changes of PVP and WHVP in Group 1 strongly suggested the existence of intrahepatic portal-systemic shunts in the normal rat liver. Opening of these shunts would permit portal blood flowing directly into the hepatic veins, leading to an elevation of WHVP and preventing further increase in PVP. When the portalvein occlusion was performed extrahepatically, the function of intrahepatic shunts was deprived, and, as a result, PVP could rise to an extremely high level. The intrahepatic shunts have not been described in the normal liver, but have been reported in cirrhotic livers in rats^[9] and in humans^[10]. It has been suggested that the frequency of large shunts (diameter > 25 μm) is relatively low and this is probably responsible for the reduction in WHVP after 80 μm microsphere injections in this study.

This model of portal hypertension is intrahepatic and can be induced rapidly, with the opening of intra- and extra-hepatic portal-systemic shunts. A major advantage of this model is that while the intrahepatic presinusoidal block is achieved acutely, the normal liver architecture remains. This may be particularly beneficial to the research in the actions of mechanical obstruction or some humoral substances, related to liver dysfunction^[2], in the pathogenesis of portal hypertension. In the experimental cirrhotic model, it is difficult to differentiate these two actions.

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