REVISTA ESPAÑOLA DE FISIOLOGIA, 40, 493-500. 1984

Splanchnic and Systemic Hemodynamic Alterations in Chronic, Progressive Portal Hypertensive Rats

A. Blanchart, M. C. Garrido D. Fernández-Muñoz, J. Nuño*, L. Hernando and J. M. López-Novoa**

Laboratorio de Fisiopatología Renal
Departamento de Nefrología
Departamento de Bioquímica Clínica
Fundación Jiménez Díaz
Madrid (Spain)

(Received on February 17, 1984)

A. BLANCHART, M. C. GARRIDO, D. FERNANDEZ-MUÑOZ, J. NUÑO, L. HERNANDO and J. M. LOPEZ-MOVOA. *Splanchnic and Systemic Hemodynamic Alterations in Chronic, Progressive Portal Hypertensive Rats.* Rev. esp. Fisiol., **40**, 493-500, 1984.

Systemic and splanchnic hemodynamics were studied by using the radioactive microsphere technique, in rats in which a chronic and progressive portal or intrahepatic hypertension was produced by the placement of a nonconstricting, well fitted ligature around the portal or suprahepatic vein when the rat weighed about 100 g. The hemodynamic measurements were performed 80-90 days after ligature placement. Suprahepatic ligated rats presented portal and intrahepatic hypertension, but nonportal-systemic shunts (PSS). The only hemodynamic disturbance observed was a decrease in renal blood flow. Portal ligated rats showed a wide range of PSS and were divided in two subgroups. The subgroups with high PSS rate (> 10 %) showed increased cardiac output and plasma renin content, as well as decreased splanchnic blood flow, portal venous inflow, hepatic blood flow and renal blood flow. Low portal-systemic shunts subgroups showed decreased cardiac output while its distribution was similar to the control rats. There was no correlation between portal pressure and shunt rate. Low shunt groups, furthermore, showed increased levels of plasma renin concentration.

Key words: Portal Hypertension, Hemodynamics, Portal-systemic shunts, Cardiac output, Splanchnic circulation.

It is conceivable that the hemodynamic changes that accompany portal hypertension can play a role in the alterations in renal function observed in patients with hepatic cirrhosis. The studies on the hemodynamic changes induced by experimental portal hypertension are scarce and, to our knowledge, none of them have been performed on a model of progressive chronic portal hypertension. Furthermore, portal hypertension in al-

* Departamento de Bioquímica Clínica, Fundación Jiménez Díaz. Madrid.

** To whom correspondence should be sent.

coholic liver disease can be either of the postsinusoidal or sinusoidal type (15). Based on these reasons, we have developed two models of progressive portal hypertension, one of presinusoidal and the other of postsinusoidal type, by placing non-constricting, non distensible ligatures around the portahepatic vein or the suprahepatic vein respectively in young rats and allowing then to grow, thus inducing a slow progressive restriction in the diameter of the vessels, and subsequently, a relative constriction of them. The present studies were therefore undertaken to determine the changes in systemic, splanchnic and renal hemodynamics induced by these two procedures.

Materials and Methods

Experiments were performed on 36 male Wistar rats of about 100 g of weigth. The animals were divided in 3 groups of 12 animals each one.

A) *Portal ligation.* Rats were anesthetized with ether, the abdomen opened in aseptic conditions by a midline incision and portal vein was exposed. A PE50 catheter was placed along side the length of the portal vein and one 000 silk ligature was tied tightly around both the catheter and the vein. Then, the catheter was removed and the portal vein was allowed to reexpand inside the ligature. This catheter size was chosen because its diameter is slightly greater than that of portal vein. Thus, no immediate constriction of the vein is produced. The viscera were placed back into the abdomen and this closed in layers with line suture. Surgical wound was treated with an antibiotic ointment and, once recovered of the anesthesia, animals were put back into the cages.

B) *Suprahepatic ligation.* After a surgical preparation similar to that described for the previous group, a 10 ml plastic syringe was placed under the back of the rat to improve the accesibility to the suprahepatic vein. Then a ligature was tied around the suprahepatic vein taking special care in placing it tightly around the vessel but without constricting it.

C) *Control group.* This group suffered the same surgical stress as the other groups except that ligature was not placed.

Hemodynamic studies were carried out 80-90 days after ligature placement or sham operation. For this purpose animals were anesthetized with nembutal, 40 mg/kg b.w., ip.

After tracheotomy, rigth carotid artery was cannulated with a PE10 tubing which, under continuous pressure monitoring was advanced until the tip reaches the left ventricle, as shown by the typical ventricular pressure curves. PE50 catheters were placed in a femoral artery for pressure measurements and blood withdrawal and a femoral vein for constant infusion of isotonic saline, 0.9 ml/h. After a small midline incision, the spleen and liver were cannulated as previously described (11) and the abdominal incision closed. Splenic, hepatic and arterial catheters were connected to pressure transducers (EM-750) and a polygraph (Lectromed MX 212), and splenic (portal), intrahepatic and arterial pressure were continuously registered.

All the surgical wounds were covered with warm, saline-moistened gauzes and the rectal temperature of the rat was controlled with a rectal probe and maintained at 37 ± 0.5° C with a heating lamp. After at least 45 min of hemodynamic stabilization, repeated measurements of aortic, hepatic and splenic pressure were performed, with the zero reference point placed at the level of the right atrium. Cardiac output (CO) and organ blood flow measurements were performed by intraventricular injection of 10^6 microspheres as pre-

viously reported (12). After measurement of femoral, hepatic and splenic pressures, a second injection of about 75,000 radioactive-labelled microspheres in 0.25 ml of saline was performed throughout the splenic catheter during 20-30 sec and this one flushed with 0.25 ml of saline. After 15 min, a sample of blood (2 ml) was obtained from the femoral catheter for biochemical determinations, the animals were killed by exanguination and the liver, kidneys, lung, small and large intestine, stomach, spleen and testes were removed, washed, weighed and counted for radioactivity in a two channel counter.

Two kinds of microspheres, 15 ± 3 μm in diameter (New England Nuclear) labeled with ^{57}Co or ^{113}Sn were used alternatively for the intraventricular and the intrasplenic injection. Adequate corrections were made for the spillover of the ^{113}Sn energy into the ^{57}Co channel. Cardiac output, organ blood flow and resistances were calculated using standard formula (13). After blood centrifugation, sodium potassium and chloride were measured using a selective electrode apparatus (Astra-4, Beckman) osmolality with an advanced osmometer; blood urea nitrogen, creatinine, total protein, direct and total bilirubin, glucose, cholesterol, gamma glutamyl transpeptidase, creatin-phosphokinase, aspartatepyruvate transaminase, glutamate-oxalacetate transaminase and alcaline phosphatase were also measured using an automathized Autoanalyzer system (SMAC-C, Technicon). Plasma renin content was measured with a radioimmunoassay. Samples of spleen and liver were fixed in formol-saline and histologically studied. Portal systemic shunt and hepatic portal flow was calculated according to CHOJKIER and GOSZMANN *et al.* (4, 8). Vascular resistances were calculated by dividing arterial pressure by blood flow. In the case of portal hepatic resistance, portal pressure was used instead of arterial pressure. To assess adequate mixing of microspheres, blood flow through left and right kidney in each rat were compared (14).

Data were statistically analyzed using analysis of variances, paired Student's t-test, and linear regression with a Hewlett-Packard Programmable calculator (model 9815 A) (16).

Results

Due to the extreme variability of the extension of portosystemic shunting, portal-ligated group has been subdivided in two subgroups, one with more than 10 % of the portal blood flowing throughout the shunts (high shunts; PHS n = 7) and other with less than 10 % of shunts (low shunts; PLS n = 5). The rat's weight at the moment of the experiment averaged 238 ± 10 g for the C group, 242 ± 14 g for the B group, 221 ± 6 g for the PHS group and 211 ± 8 g for the PLS group without significant difference between them.

Systemic hemodynamics are represented in table I. PHS group shows a CO higher than controls whereas PLS have a CO lower than controls. Also total peripheric resistances are slightly lower than controls in PHS and slightly higher in PLS group. The differences were not significant when compared with control animal but clearly significant when compared both portal ligated groups. Both suprahepatic ligated and high shunts, portal ligated rat groups showed a fall in renal perfusion, mainly based on an increase in renal vascular resistance. Renal Blood Flow (RBF) is similar for both kidneys in all the animals studied.

Portal-hepatic hemodynamic data are shown in table II. All the experimental

Table I. *Systemic hemodynamics.*

	Control	Suprahepatic ligation	Portal vein ligation high shunts	Portal vein ligation low shunts
Cardiac output ml × min^{-1} × 100^{-1}	28.23 ± 1.82	25.04 ± 2.28	32.35 ± 3.18*	22.79 ± 2.03*[a]
Arterial pressure, mmHg	108.7 ± 4.1	102.5 ± 4.81	103.4 ± 3.0	108.4 ± 4.0
Total peripheral resistance mmHg × ml^{-1} × min × 100 g	4.03 ± 0.34	4.37 ± 0.33	3.40 ± 0.42	4.92 ± 0.58[a]
Renal blood flow ml × min^{-1} × g^{-1}	6.47 ± 0.58	4.93 ± 0.50*	4.15 ± 0.61*	5.94 ± 1.20
Renal vascular resistance mmH × ml^{-1} × min × g	18.06 ± 1.52	22.37 ± 1.83*	27.75 ± 3.89*	23.17 ± 8.12
Stroke volume μl × beat^{-1}	183.6 ± 16.0	170.7 ± 13.5	187.7 ± 12.9	136.9 ± 18.4*[a]
Heart rate beats × min^{-1}	354.4 ± 10.2	357.2 ± 6.35	377.3 ± 12.1	354.7 ± 11.4

* $p<0.05$ respect to control rats; [a] $p < 0.05$ respect to high shunts group.

groups show increase in portal pressure and in intra-hepatic pressure. Portal venous inflow is decreased in high shunts, portal-ligated rats, and maintained in the other groups. Also PHS group shows decreased portal-hepatic blood flow and total hepatic blood flow, but it keeps hepatic arterial blood flow at normal levels.

Portalsystemic shunts appear only in portal ligated rats. There was no correlation between portasystemic shunt flow

Table II. *Porta-hepatic hemodynamics.*

	Control	Suprahepatic ligation	Portal vein ligation high shunts	Portal vein ligation low shunts
Portal venous inflow ml × min^{-1}	8.96 ± 0.94	8.30 ± 0.62	5.90 ± 1.07*	8.61 ± 1.64[a]
Portal pressure cm H_2O	8.05 ± 1.12	15.13 ± 1.63*	23.87 ± 4.59*	15.50 ± 2.08
Portal hepatic blood flow Ml × min^{-1} × g^{-1}	1.56 ± 0.17	1.28 ± 0.10	0.37 ± 0.30*	1.48 ± 0.29[a]
Portal hepatic resistance mmHg × ml^{1} × min × g	6.30 ± 1.15	12.97 ± 7.94*	321.02 ± 10.50*	11.27 ± 1.53*[a]
Hepatic arterial blood flow ml × min^{-1} × g^{-1}	0.62 ± 0.09	0.83 ± 0.17	0.61 ± 0.08	0.52 ± 0.11
Hepatic arterial resistance mmHg × ml^{-1} × min × g	216.4 ± 31.0	198.8 ± 57.1	175.7 ± 51.2	258.8 ± 98.7
Intrahepatic pressure cm H_2O	4.96 ± 0.65	15.61 ± 2.68*	12.5 ± 3.43*	8.32 ± 2.14*
Hepatic blood flow ml × min^{-1} × g^{-1}	2.16 ± 0.22	2.02 ± 0.18	1.02 ± 0.28*	2.00 ± 0.39[a]
Portal systemic shunting % (Portal venous inflow)	0.23 ± 0.09	0.52 ± 0.30	78.10 ± 12.41*	2.01 ± 1.80*[a]

*$p < 0.05$ respect to control rats; [a] $p < 0.05$ respect to high shunts group.

Table III. *Splanchnic hemodynamics.*

	Control	Suprahepatic ligation	Portal vein ligation high shunts	Portal vein ligation low shunts
Total splanchnic blood flow ml × min^{-1}	12.42 ± 1.92	13.38 ± 1.75	8.99 ± 1.12*	11.13 ± 1.9
Gastric blood flow ml × min^{-1}	0.46 ± 0.02	0.47 ± 0.05	0.49 ± 0.1	0.44 ± 0.05
Large intestine blood flow ml × min^{-1}	1.16 ± 0.11	1.05 ± 0.09	1.00 ± 0.13	1.19 ± 0.22
Small intestine blood flow ml × min^{-1}	6.03 ± 0.79	5.03 ± 0.66	3.56 ± 0.78*	4.98 ± 1.13
Splenic blood flow ml × min^{-1}	1.29 ± 0.23	1.06 ± 0.26	0.78 ± 0.28*	1.47 ± 0.58[a]

* $p < 0.05$ respect to control rats; [a] $p < 0.05$ respect to high shunts group.

rate and portal pressure or cardiac output in any group.

Splanchnic hemodynamics are represented in table III. PHS group shows decreased splanchnic blood flow, mainly due to reduction in small intestine, splenic and hepatic arterial blood flow. Histologically, there were no signs of hepatic damage in any of the animals studied. A small increase in hepatocyte size with decrease in Disse space was observed in high-shunts, portal ligated rats, but detailed quantitative morphometric studies were not performed. This group showed also increased levels of aspartate aminotransferase (564 ± 94 U/ml, vs. 307 ± 61 mU/ml in control group) alkaline phosphate (245 ± 64 mU/ml vs 121 ± 30 mU/ml in control group) and plasma renin content (310.8 ± 81.0 vs 119.4 ± 19.2 ng AI / ml / h in control group). All the other biochemical parameters measured were not different in any group from control data.

Discussion

The main finding of our study is the demonstration that hemodynamic changes associated with portal hypertension are different depending on the extension of portal-systemic shunt development, and that this development depends on the site of ligature placement. Another meaningful result is that portal hypertension with high shunting rate and suprahepatic ligation are associated with decreases in renal blood flow without fall in arterial pressure.

Major points of this paper in relation to the results previously published in the acute model of portal ligation (2 -4,8) are: a) shunt formation: In the paper from GROSZMANN'S group, portal-systemic shunting averages 96 % of the portal inflow (3, 4) whereas in the paper of BLANCHET and LEBREC (2) shunting can be calculated to average 26 % of the portal blood flow. Our data show a great variability in the percentage of shunting, which ranges between 0.08 and 99.6 % of the portal flow. This observation induced us to divide the portal ligated group into two subgroups: One with high shunt rate and the other with low shunt rate. These two subgroups present clear differences: cardiac output is increased in the group with high shunts, which agree with previously reported data (2, 3, 4). However, CO is decreased in portal-ligated rats with low shunts percentage. Renal blood flow is maintained in low shunt groups.

b) Portal venous inflow was reported in previous papers to be increased in acute portal hypertension, in presence of extensive shunt formation (2, 4). In contrast our results report decreased portal venous inflow in high shunt group, and normal portal inflow in low shunt group. Similarly, BLANCHET and LEBREC found that portal-ligated rats showed increased intestinal and splenic blood flow (2) whereas our data reveal that small intestine and splenic blood flow are decreased in high shunt groups and maintained in low shunt groups.

c) As opposite to acute portal constriction, which has been reported to increase hepatic arterial blood supply (2, 4) this parameter does not change with the progressive constriction.

Increase in cardiac output usually accompanies clinical and experimental portal hypertension and liver cirrhosis (9). Our results seem to demonstrate that this increased cardiac output is more related to the presence of portal-systemic shunts than to the portal hypertension *per se*. This hyperdynamic state has been attributed to the fact that portal-systemic shunts act like an arterio-venous fistule (2, 3). However, we have demonstrated that cirrhotic rats with very low shunts percentage also present high cardiac output (7). The common mechanism could be the presence in plasma of vasoactive substances of intestinal origin and hepatic catabolism that could bypass the liver through the shunts or not be catabolized by the cirrhotic liver and induce peripheral vasodilatation, increase venous return and subsequently, increase cardiac output. However, this hypothesis is highly speculative, and the mechanism of the circulating hyperdynamic state remains to be solved.

The fact that rats with the higest cardiac output show a decreased blood flow throughout the kidneys and the splanchnic organs, the ones that usually drain most of cardiac output, is highly surprising, and we have not a definitive explanation for it. However, the above exposed mechanism for the increased cardiac output, could also be applied. Thus, the extensive decrease in peripheral resistances could be responsible for draining most of cardiac output, thus producing a slight hypotension and stimulating renin secretion.

The high levels of plasma renin shown by these animals could explain, at least in part, the decrease in renal blood flow. This decrease can be also explained by the increased sympathetic stimulation caused by intrahepatic hypertension (1). This stimulation has been demonstrated to increase intrarenal vascular resistance and, subsequently, reduce renal blood flow (1). This decrease in RBF has also been observed in our portal-ligated rats with high shunt rates. This increase in renal neural activity has been recently reported to be responsible for some of the physiopathological alterations observed in the cirrhosis of the liver and in the hepatorenal syndrome (5).

Both suprahepatic and portal ligature have been reported to be associated with impairment of the renal function and salt and water retention (1, 10). Even if in this paper we have not specifically dealt with this problem it is possible that the reduction in renal blood flow observed in these two models could be in some way involved in this impairment.

The results above presented also suggest that shunt formation does not only depend on the portal pressure, because there is no correlation between these two parameters. In addition, suprahepatic ligated rats, that also develop portal hypertension, never did show portal-systemic shunting.

Chronic suprahepatic constriction induces marked increases in intrahepatic and portal pressure without shunt for-

mation changes in cardiac output and splanchnic hemodynamics or the formation of significant amount of ascites. It has been suggested that ascites formation due to post-sinusoidal hypertension requires also almost complete blocking of hepatic venous outflow, derangement of hepatic function or changes in the physicochemical properties of the blood (e.g.: decreased oncotic pressure due to hypoalbuminenia) (6) and none of these facts are present in our postsinusoidal hypertensive rats.

From the above exposed, it can be deduced that chronic progressive portal hypertension induces extensive hemodynamic changes which depend mainly on the placement of the ligature and the extent of portal-systemic shunts. The mechanism that mediates hemodynamic alterations remains to be elucidated.

Acknowledgements

The authors acknowledge the collaboration of Dr. J. Cortés (Dept. of Pathology, «Fundación Jiménez Díaz») in the study of the histological samples, Liselotte Gulliksen kindly typed the manuscript. A. Blanchart is a fellow of the «Fondo de Investigaciones Sanitarias de la Seguridad Social» (FIS), National Institute of Health of Spain (INSALUD). D. Fernández-Muñoz is a fellow of the Iñigo Alvarez de Toledo Foundation. This paper was supported in part by a grant from FIS (INSALUD) and the Iñigo Alvarez de Toledo Foundation.

Resumen

Se estudia la hemodinámica sistémica y esplánica en ratas de unos 100 g de peso, que previamente se ha inducido hipertensión portal o intrahepática crónica y progresiva mediante la colocación de una ligadura inicialmente no constrictiva alrededor de la vena porta o suprahepática respectivamente.

Las medidas hemodinámicas se realizan entre 80 y 90 días después de la colocación de la ligadura utilizando la técnica de las microesferas radioactivas. Las ratas con ligadura suprahepática presentan hipertensión portal e intrahepática, pero no cortocircuitos portosistémicos y disminución del flujo sanguíneo renal. Las ratas con ligadura en la vena porta muestran un amplio rango de cortocircuitos portosistémicos (entre el 0,08 y el 99,6 % del flujo portal): un grupo con alto porcentaje de cortocircuitos (> 10 %) presenta gasto cardíaco y renina plasmática aumentada y disminución en el flujo sanguíneo hepático, esplácnico y renal, y un grupo con bajo porcentaje de cortocircuitos con el gasto cardíaco disminuido, y su distribución es similar al de las ratas controles. No hay correlación entre la presión portal y el porcentaje de cortocircuitos.

References

1. Anderson, R. J., Cropin, R. E., McDonald, K. and Schrier, R. W.: *J. Clin. Invest.*, 58, 964-970, 1976.
2. Blanchet, L. and Lebrec, D.: *Eur. J. Clin. Invest.*, 12, 327-330, 1982.
3. Bredfeldt, J. E., Vorobioff, J. and Groszmann, R. J.: *Hepatology*, 1, 499 (a), 1981.
4. Chojkier, M. and Groszmann, R. J.: *Am. J. Physiol.*, 240, G371-G375, 1981.
5. Dibona, G.: *Kidney Int.*, 25, 841-853, 1984.
6. Epstein, M.: *Gastroenterology*, 76, 622-635, 1979.
7. Fernández-Muñoz, D., Caramelo, C., Blanchart, A., Santos, J. C., Hernando, L. and López-Novoa, J. M.: *Eur. J. Clin. Invest.*, 14, 48 (a), 1984.
8. Groszmann, R. J., Vorobioff, J. and Ridley, E.: *Am. J. Physiol.*, 242, G156-G16, 1982.
9. Kontos, H. A., Shapiro, W., Maur, H. P. and Patterson, J. L.: *Am. J. Med.*, 37, 526-535, 1964.
10. Levy, M.: *Am. J. Physiol.*, 227, 1084-1087, 1974.
11. López-Novoa, J. M.: *Experientia*, 37, 536-537, 1981.
12. López-Novoa, J. M., Ramos, Martín-Oar, J. E. and Hernando, L.: *Renal Physiol.*, (Basel), 5, 76-84, 1982.
13. Malik, A. G., Kaplan, I. E. and Sabre, T. M.: *J. Appl. Physiol.*, 40, 472-475, 1976.
14. McDevitt, D. G. and Nies, A. S.: *Cardiovasc. Res.*, 10, 494-498, 1976.
15. Sherlock, S.: *Am. J. Surg.*, 127, 121-127, 1974.
16. Sokal, R. R. and Rolf, F. R.: Biometry, H. Blume, Madrid, 1979.