Altered Proteinogram in Short Term Portal Vein Stenosed Rats

M.P. Nava[1], M.A. Aller[2], M. Vega[3], I. Prieto[2], F. Valdes[4], J. Arias[2]

[1]*Animal Physiology II Department, Biology Faculty, Complutense University, Madrid*
[2]*Surgery Department I, Medical Faculty, Complutense University, Madrid*
[3]*Angiology and Vascular Surgery Service, Hospital Clinico San Carlos, Madrid*
[4]*Clinical Analyses Service, Hospital Clinico San Carlos, Madrid*
Spain

Abstract

The electrophoretic pattern of serum proteins has been studied in short-term prehepatic portal hypertensive rats since atrophy is produced in the liver, which is the main origin of most of these proteins, during this postoperative period.

After 28 days of evolution, rats (n = 9) with triple stenosing ligated portal vein showed hypoalbuminemia, hypo-α-globulinemia, hyper-α_2-globulinemia and hyper-γ-globulinemia, the albumin/globulin ratio decreased with respect to the control animals (n = 8). These alterations are associated with hepatic atrophy, portosystemic and portohepatic (44.4%) collateral circulation.

The proteinogram alterations found in rats with short-term prehepatic portal hypertension suggest that hepatic failure exists in spite of potential portohepatic revascularization which is frequently originated by the development of portohepatic collateral circulation.

Key Words: portal hypertension, proteinogram, rat

Introduction

Portal hypertension is a clinical syndrome which is usually originated by an intra- or extrahepatic obstruction of portal flow. It is characterized by a pathological increase of portal vein pressure, associated to splenomegaly and by an increase of portosystemic collateral circulation which directs portal flow to the systemic circulation bypassing the liver (3, 23).

However, it has been suggested that a highly variable degree of portosystemic shunts is produced in human prehepatic portal hypertension by portal vein obstruction and that many of the shunts could be portoportal ones that serve to bridge the obstructed segment which is why hepatic atrophy is usually not present (17).

The rat experimental model usually employed to study prehepatic portal hypertension is obtained by simple stenosing portal vein ligation (4, 24) although the alterations are much more homogeneous than those described in human prehepatic portal hypertension because there is a narrow range of portal hypertension, grade of portosystemic shunts and hepatic atrophy (11). However, this evolutive uniformity cannot be verified from our previous studies using a modified technique of portal vein calibrated stenosis in the rat since the degree of hepatic atrophy and the portosystemic collateral circulation developed are variable (14, 20). In addition, as in human prehepatic portal hypertension, long-term portoportal circulation is developed, but does not always avoid hepatic atrophy (1).

Since the portal revascularization of the liver in experimental portal prehepatic hypertension could revert their functional alterations, the aim of this

Corresponding author: to Dr. M. P. Nava, Dpto. Fisiología Animal II, Facultad de Biología, Universidad Complutense, 28040 MADRID (Spain), Tel.: 91-3944983, Fax: 91-3944935, e-mail: mpaznava@bio.ucm.es
Received: February 20, 2002; Revised: May 6, 2002; Accepted: May 18, 2002.

work was the study of the electrophoretic pattern of rat serum proteins after 28 days of portal hypertension and the developing of portosystemic and portohepatic collateral circulation, as the liver is the main origin of most of these proteins (9, 10, 12, 13, 19).

Materials and Methods

Animals and Experimental Design

Male Wistar rats from the *Vivarium* of the Complutense University in Madrid were used in this experiment. Their initial body weights ranged from 236 g to 248 g. The animals were divided into two groups: control group (n = 8) formed by intact animals (no anaesthesia and no surgery) and triple stenosing ligated portal vein group (n = 9). After 28 days of evolution, the animals were anesthetized and blood was drawn from their aorta. Final body (BW), liver (LW), spleen (SW), kidney (KW) and testes (TW) weights were determined in both groups.

Triple Stenosing Ligature of the Portal Vein (TSLP)

The rats were anesthetized with an i.m. injection of ketamine (75 mg/kg) and diazepam (5 mg/kg) and a midline abdominal incision was made. After visualizing and dissecting the portal vein, prehepatic portal hypertension was produced by triple stenosing ligation. Three stenosing ligatures were performed in the superior, medial and inferior portions of the portal vein and maintained in position by the previous fixation of the ligatures to a silastic guide. The stenoses were also calibrated by a simultaneous ligature (3-0 silk) around the portal vein and a 20 G needle. It has been suggested that the initial increase in portal pressure that immediately follows portal vein stenosis may have permanent effects on the extent of collateral development. If so, the greater initial increase in portal pressure in rats with triple portal vein stenosis could worsen the evolution of the portal hypertension (8). The abdominal incision was closed on two layers with catgut and 2-0 silk.

Portosystemic Collateral Circulation and Venous Mesenteric Vasculopathy

The areas of developed collateral venous circulation splenorenal (superior cranial and inferior caudal), gastroesophageal, colorectal and hepatic hilum were exposed with a midline abdominal incision to study the existence and pathways of increased collateral veins (1, 14, 20). Mesenteric vasculopathy due to splanchnic hypertension was graded in order to make a better quantification (Grade 0: normal development of branches of the superior mesenteric vein and negative to the Manouver of Pringle, Grade I: dilatation and tortuosity of those branches as a response to the handling of Pringle, and Grade II: spontaneous dilatation and tortuosity of branches of the mesenteric vein).

All the experimental procedures used in this work were in accordance with the Guidelines for the Care and Use of Laboratory Animals (1986) published in Spain in RD 223/1988.

Electrophoresis

Blood collected from rats of both groups was centrifuged (3,500 r.p.m., 15 min) and plasma samples frozen until electrophoresis was performed on acetate/ cellulose (Olympus High System 600). Total proteins were measured by refractometry (Clinical Refractometer Erma).

Statistical Analysis

The data are expressed as the mean ± standard deviation (S.D.). A Student-*t* test for unpaired data were used for the statistical comparison. The results are considered statistically significant if $p < 0.05$.

Results

Body and Organ Weight

Table 1 shows the decreased ($p < 0.05$) final body weight of rats with TSLP. With respect to the organ weights, those of the liver and spleen were significantly increased ($p < 0.01$) in the experimental rats. In the same group, the testes and kidney weights were lower but not significantly.

Collateral Circulation and Mesenteric Venous Vasculopathy

Portosystemic collateral circulation developed in all the animals with TSLP, however, the percentages regarding the sites were different: 22.2% (n = 2) paraesophageal, 55.5% (n = 5) pararectal and 88.8 % (n = 8) splenorenal. Portohepatic collateral circulation, represented by the accessory hepatic vein, was observed in 44.4% of the animals (n = 4) with TSLP.

Grade I (n= 3; 33.3%) or grade II (n = 6; 66.6%) mesenteric venous vasculopathy appeared in all the animals belonging to the experimental group.

Serum Proteins Concentration

Although the total proteins values for the control and TSLP rats were not significant, the remaining

Table 1. Body and Several Organ Weights of Control and Triple Stenosing Ligature of the Portal Vein (TSLP) Rats at 28 Days of Postoperative Evolution

	CONTROL (n = 8)	TSLP (n = 9)
Weights (g)		
• Initial body	243.55 ± 5.38	240.87 ± 3.35
• Final body	342.73 ± 21.51	318.40 ± 20.80*
• Body increase	99.18 ± 23.92	77.53 ± 20.64
• Liver	9.76 ± 1.84	7.76 ± 0.85*
• Spleen	0.59 ± 0.08	0.78 ± 0.16**
• Testes	3.27 ± 0.26	3.08 ± 0.14
• Kidneys	2.06 ± 0.19	1.84 ± 0.30

Values are mean ± S.D.; *p < 0.05, **p < 0.01.

Table 2. Albumin, Globulins (α_1, α_2, β and γ) and Total Serum Proteins in Control and Triple Stenosing Ligature of the Portal Vein (TSLP) Rats at 28 Days of Postoperative Evolution

	CONTROL (n = 8)	TSLP (n = 9)
Total proteins (g/dl)	5.64 ± 0.26	5.39 ± 0.29
Albumin		
g/dl	3.29 ± 0.16	3.05 ± 0.21*
%	58.31 ± 0.01	56.61 ± 1.39**
Albumin/Globulin	1.40 ± 0.09	1.31 ± 0.07*
α_1-globulin		
g/dl	0.86 ± 0.09	0.71 ± 0.06***
%	15.30 ± 0.01	13.41 ± 1.34**
α_2-globulin		
g/dl	0.29 ± 0.04	0.41 ± 0.04***
%	5.19 ± 0.01	7.46 ± 1.19***
β-globulin		
g/dl	0.93 ± 0.10	0.86 ± 0.11
%	16.40 ± 0.02	16.08 ± 0.87
γ-globulin		
g/dl	0.26 ± 0.05	0.35 ± 0.05**
%	4.88 ± 0.01	6.44 ± 0.9***

Values are mean ± S.D.; *p < 0.05, **p < 0.01; ***p < 0.001.

values except, for that of β-globulin, showed statistically significant differences (Table 2). Values for albumin ($p < 0.05$) and α_1-globulin ($p < 0.001$) are higher in control animals when compared to TSLP animals. On the contrary, α_2 ($p < 0.001$) and γ-globulin ($p < 0.01$) showed higher concentrations in the TSLP group.

Discussion

The model of prehepatic portal hypertension after portal vein stenosis in the rat is characterized by its evolutive variability and it has been demonstrated that mechanisms originating portal hypertension differ in their different phases (24, 25). After one month of evolution it is considered that the increased portal pressure is caused by the increased portal venous inflow and by the high resistance offered by the portal collaterals to that flow (24, 25). Therefore, a relationship has been established between the splanchnic hyperdynamic circulation (forward flow) and the degree of portosystemic shunting (backward flow) needed to maintain the splanchnic venous pressure elevated (25).

However, the finding of portoportal collateral circulation in rats with TSLP in that evolutive phase suggests that early liver portal revascularization occurs and therefore this type of collateral circulation could participate in the regulation of portal pressure as well as in the reversion of the hepatic atrophy that follows portal stenosis. Since hepatic portal revascularization through the hepatic accessory vein occurs in 44.4% of the cases, the incidence of this factor could influence the individual evolution of rats with short-term portal hypertension, particularly with respect to the degree of hepatic atrophy (14, 20). The appearance of mesenteric venous vasculopathy in all the animals with TSLP has been considered as a signal of splanchnic venous flow increase, as it is associated to an increment in the number and diameter of intestinal submucosal vessels and in the number of mast cells (6).

The electrophoretic pattern alterations of serum proteins in rats with one month prehepatic portal hypertension are similar to those described in chronic hepatic failure (9, 10, 18) because albumin and α_1-globulins concentrations decrease, γ-globulins concentrations increase and the albumin/globulin ratio decreases (Table 2). Hypoalbuminemia may occur in portal vein-stenosed rats as a consequence of the reduction of hepatic synthesis secondary to hepatocellular damage after deprivation of portal flow, although it could also be a consequence of abnormal distribution and catabolic increase. In this sense, the inflammatory hypothesis proposed to explain the alterations related with experimental portal hypertensive enteropathy (5, 6) would involve the possibility of episodes with a sudden release of inflammation mediators from mast cells that would cause plasma exudation response. In addition, the inflamed gut mucosa could originate plasma protein loss into the gut or protein losing gastroenteropathy and the resulting hypoalbuminemia would be a

predisposing factor to the development of the intestinal edema.

α_1-globulins decrease could be secondary to the α_1-antitrypsin deficiency as it forms 90% of this fraction of plasmatic proteins. The decrease of circulating α_1-antitrypsin makes the action of proteolytic enzymes possible and it has also been reported in humans due to protein-losing enteropathy and in association with intestinal mucosal atrophy (7, 8). Since changes in α_2 and β globulins reflect lipoproteins alterations, it is difficult to explain the increase of α_2-globulins in rats with prehepatic portal hypertension, although it has not been discarded that it could be caused by the larger production of acute phase proteins. Thus, plasmatic TNF-α increase in this experimental model associated with hypercortisolemia (15) makes up two factors that stimulate the hepatic secretion of acute-phase proteins (16).

Hyper-γ-globulinemia in rats with portal prehepatic hypertension could be due to humoral antibody production after intestinal antigenic and bacterial stimulus. The existence of intestinal bacterial translocation to mesenteric lymphatic nodes which, at the same time, show an increase of infiltration by mast cells, has been demonstrated in these animals (2). It is possible that the permeability alteration of the intestinal mucosal barrier, which has an inflammatory etiology (6), favors the intestinal translocation of bacteria and bacterial antigens into both lymphatic and portal venous system, reaching the systemic circulation bypassing the liver and providing a stimulus to γ-globulin production. The existence of splenomegaly in association with portal hypertension also indicates that the spleen is involved in the immune response as it increases its phagocytic capacity (22) and the production of antibodies by B cells. In addition, the strong antibody response could be mediated by type 2 T helper (Th_2) cells, a polarized form of the CD_4^+Th cell-mediated immune response (21).

In summary, early prehepatic portal hypertension and the alterations of the electrophoretic pattern of serum proteins in the rat are suggestive of hepatic failure and are associated with liver atrophy, although portohepatic collateral circulation is frequently developed. These results make it possible to suspect that portal hepatic revascularization by means of the hepatic accessory vein performs an inhibitory effect on the hepatic trophism, perhaps due to the supply of factors that either inhibit regeneration or favor apoptosis and that are originated in the splanchnic inflamed region .

Acknowledgements

This work was partially supported by a UCM grant, Ref. No. PR52/00-8898.

References

1. Aller, M.A., Nava, M.P., Palma, M.D., López, L., Arias, J.L., Vara, E., Arias, J. Redistribución del peso lobular hepatico en ratas con hipertensón portal prehepática crónica. *Cir. Esp*. 70: 235-241, 2001.
2. Alonso, M.J., Aller, M.A., Ramos, C., Corcuera, M.T., Nava, M.P., Casado, I., Gomez-Lus, M.L., Prieto, J., Arias, J. Increased mesenteric lymph node infiltration by mast cells in triple-ligated portal hypertensive rats. *Rev. Esp. Inv. Quir*. 4: 188-194, 2001.
3. Bosch, J, Garciá-Pagán, J.C. Complications of cirrhosis. I. Portal hypertension. *J. Hepatol.* 32 (1 Suppl): 141-156, 2000.
4. Chojkier, M, Groszman, R.J. Measurement of portal systemic shunting in the rat by using gamma-labelled microspheres. *Am. J. Physiol*. 240: G371-G375, 1.981.
5. Díez-Arias, J.A., Aller, M.A., Nava, M.P., Gragera, R., Diéguez, B., Muñiz, E., Arias, J. Portal hypertensive duodenopathy in the rat. *Rev. Esp. Inv. Quir*. 4: 25-29, 2001.
6. Díez-Arias, J.A., Aller, M.A., Palma, M.D., Arias, J.L., Muñiz, E., Sánchez, M., Arias, J. Increased duodenal mucosa infiltration by mast cells in rats with portal hypertension. *Dig. Surg*. 18: 34-40, 2001.
7. Fisher, R.L., Taylor, L., Sherlock, S. α1AT deficiency in liver disease: the extent of the problem. *Gastroenterology* 71: 646-651, 1976.
8. Greenwald, S.J., Johnson, D.S., Oskvig, R.M., Aschenbrener, C.A., Randa, D.C. α1AT deficiency, emphysema cirrhosis and intestinal mucosal atrophy. *J. Am. Med. Asoc*. 231: 273-276, 1975.
9. Hasch, E., Jarnum, S., Tygstrup, N. Albumin synthesis as a measure of liver function in patients with cirrhosis. *Acta Med. Scand*. 182: 83-92, 1967.
10. Levin, A.S., Jeffay, H. Metabolism of serum albumin in rats with cirrhosis of the liver. *J. Lab. Clin. Med*. 63: 776-783, 1964.
11. Lin, H-C., Yang, M.C-M., Hou, M-C., Li, S-M., Huang, Y-T., Yu, P-C., Tsai, Y-T., Lee, S-D. Effects of long-term administration of octreotide in portal vein-stenosed rats. *Hepatology* 23: 537-543, 1996.
12. Miller, L.L., Bly, C.G., Watson, M.L., Bale, W.F. The dominant role of the liver in plasma protein synthesis. A direct study of the isolated perfused rat liver with the aid of lysine-εC^{14}. *J. Exp. Med*. 94: 431- 453, 1951.
13. Miller, L. L. and Bale, W. Synthesis of all plasma protein fractions except gamma globulins by the liver. *J. Exp. Med*. 99: 125-132, 1954.
14. Monterde, G., Cuadrado, M.A., Aller, M.A., Nava, M.P., Lorente, L., Arias, J. Different evolution of the liver weight in prehepatic portal hypertension rats. *Rev. Esp. Inv. Quir*. 2: 85-88, 1999.
15. Monterde, G., Rodríguez-Fabián, G., Vara, E., López, L., Arias, J., Aller, M.A., Arias, J. Increased plasma levels of corticosterone and prolactin and decrease T_3 and T_4 levels in short-term prehepatic portal hypertension in rats. *Dig Dis Sci* 45: 1865-1871, 2000.
16. O'Connor, T.M., O'Halloran, D.J., Shanahan, F. The stress response and the hypothalamic pituitary-adrenal axis: from molecule to melancholia. *Q. J. Med*. 93: 323-333, 2000.
17. Pak, J.M., Lee, S.S. Glucagon in portal hypertension. *J. Hepatol*. 20: 825-832, 1994.
18. Price, C.P., Albert, K.G.M.M. Biochemical assessment of liver function. In: Liver and biliary diseases. Pathophysiology. Diagnosis. Management, edited by Wright, R., Alberti, K.G.M.M., Karran, S., Millward-Sadler, G.H. London: W.B. Saunders Co. Ltd. 1979, 17: 381-416.
19. Redman, C.M., Cherian, M.L. The secretory pathways of rat serum glycoproteins and albumin: localization of newly formed proteins within the endoplasmic reticulum. *J. Cell Biol*. 52: 231-245, 1972.

20. Rodríguez-Fabián, G., Monterde, G., Diéguez, B., Aller, M.A., Arias, J. Hipertensión portal a largo plazo en la rata por triple ligadura estenosante de la vena porta. *An. Med. Interna* 17: 137-141, 2000.
21. Romagmani, S. T-cell subsets (Th1 versus Th2). *Ann. Allergy Asthma Immunol*. 85: 9-21, 2000.
22. Rozga, J., Jeppsoon, R., Hägerstrand, I., Bengmark, S. Acute portal vein stenosis. *Acta. Chir. Scand*. 151: 125-131, 1985.
23. Sherlock, S. The portal venous system and portal hypertension. In Diseases of the liver and biliary system (8ª ed). *Oxford. Blackwell Scientific Publications*, 1989, pp. 151-207.
24. Sikuler, E., Kravetz, D., Groszman, R. J. Evolution of portal hypertension and mechanisms involved in its maintenance in a rat model. *Am. J. Physiol*. 248: G618-G625, 1.985.
25. Sikuler, E., Groszman, R.J. Hemodynamic studies in long- and short-term portal hypertensive rats: the relation to systemic glucagon levels. *Hepatology* 6: 414-418, 1.985.