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Increased Plasma Volume in Two Models of Portal Hypertension in the Rat: Cirrhosis of the Liver and Partial Portal Vein Ligation

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Portal hypertension has been studied in the rat to see if it is associated to altered blood volume composition, as it has been shown in other species. Plasma volume was measured by isotope dilution using ^{99m}Tc labelled albumin in three groups of male Sprague-Dawley rats: normal rats (controls), partially ligated portal vein rats and rats with Cl_4C induced cirrhosis. Plasma volume was significantly higher in rats with portal hypertension due to partially ligated portal vein and cirrhosis than in control animals. Similarly, the calculated blood volume was also significantly higher in the portal hypertensive animals than in control group. Portal hypertension in the rat, therefore, has been demonstrated to be associated to a marked hypervolemia and this finding should be taken into consideration in haemodynamic and pharmacokinetic studies in portal hypertensive rat models.

Key words: Portal hypertension, Blood volume.

Portal hypertension is a common syndrome with important clinical consequences. It is well known that the plasma volume is markedly increased in patients with portal hypertension (5, 17). This has also been observed in cirrhotic dogs (15, 16). Recently different models of portal hypertension have been developed in the rat for the study of drug pharmacokinetics (4), ascites formation (11), systemic and splanchnic haemodynamics (20, 22, 23) and the circulatory changes produced by haemorrhage and blood volume restitution (13). Although the plasma volume is an important parameter in these studies, there is no information on whether it is normal or increased, and to what extent, in rats with portal hypertension. For this reason the present inves-

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tigation was aimed at studying the plasma volume in the two currently used models of portal hypertension in the rat.

Materials and Methods

The study was performed in 31 Sprague-Dawley rats (Panlab SL. Spain), divided in 3 groups. A control group of normal rats (n = 12, weight: 330 ± 16 g) and two groups of rats with portatension, group 1: partial portal vein ligation (PPVL, n = 10, weight: 309 ± 8 g) group 2: rats with histologically proven cirrhosis of the liver, (n = 9, weight: 389 ± 8 g). The procedure for the production of portal hypertension in the PPVL rats has been previously described (8). In brief, the portal vein was isolated and the stenosis created by a single ligature of 3-0 silk around the portal vein and a 20 gauge blunt tipped needle. The needle was then removed. The plasma volume determination was performed 2 weeks after this procedure, when the portal hypertension syndrome is fully developed (20, 22).

Cirrhosis of the liver was induced by carbon tetrachloride following the method described by MACLEAN *et al.* (19) and modified by LÓPEZ-NOVOA *et al.* (18). The rats received phenobarbital diluted in the drinking water (0.3 g/l) starting 1 week before carbon tetrachloride administration in order to shorten the time required to induce cirrhosis. Carbon tetrachloride was administered by inhalation twice a week for increasing periods (1 to 5 min). For this the rats were placed in a special chamber and compressed air was passed via flowmeter bubbling through a flask containing carbon tetrachloride and into the box (18). After 12-14 weeks, when all rats had developed cirrhosis, the animals were studied. The rats were fed on a complete laboratory diet and received water *ad libitum* until the moment of the study.

Measurements of plasma volume were performed using human serum albumin (Thechnescon HSA, MallincKrodt, 50 mg/vial) labelled with ^{99m}Tc (^{99m}Tc-HSA) as indicator. The preparation of ^{99m}Tc-HSA was done adding 5 ml of isotonic saline with 1 mCi (3.7×10^7 Bq) of $^{99m}TcO^-_4$ (Techenetium ^{99m} generator, Amertech 11, Amersham), to the human albumin vial (specific activity 20 Ci/mg, $7{,}4 \times 10^5$ Bq/mg). After 15 min of incubation the labelling efficiency was higher than 99%, determined by Whatman 31 ET paper acetone solvent and international thin layer chromatography-silicagel (ITLC-SG; Gelman Instrument Co.), media (saturated with HSA), ethyl alcohol, ammonium hydroxide water (2:1:5) solvent. The femoral artery and femoral vein were cannulated under Ketamine anesthesia (100 mg/kg/bw, im), using PE-50 catheters. Rats were injected via femoral vein with 200 μl of ^{99m}Tc-HSA. Blood samples of about 0.5 ml were obtained from the femoral artery 10 min after the injection, collected in heparinized tubes, and centrifuged at 10,000 rpm for 10 min. Triplicate samples of 100 μl of plasma were used for radioactivity measurements cpm/ml (21). A standard was prepared by diluting 200 μl of ^{99m}Tc-HSA with 20 ml of isotonic saline. All the measurements were performed using a Packard 800 C Counter. Duplicate measurements of packed cell volume (PCV) were obtained after centrifugation for 5 min at 11,000 × g and corrected for trapped plasma (7). Plasma volume (PV) was calculated by dividing the cpm of the total injected ^{99m}Tc-HSA dose by the cpm/ml of the plasma obtained at 10 min multiplied by 1.015 (correction factor) (10). The blood volume was obtained by the equation PV × 100/100-PCV and the red blood cell (RBC) volume as the difference between blood volume and PV.

After completing these measurements, an abdominal midline incision was performed and the portal vein was cannulat-

Table I. *Blood volumes (ml/100 g bw) in normal and portal hypertensive rats.*
Values are Means ± SEM. n: number of rats. Hct: Hematocrit. RCB volume; red blood cell volume.

Group	n	Hct. (%)	Plasma volume	RCB volume	Blood volume
Normal rats	12	44.9 ± 0.9	4.0 ± 0.1	2.7 ± 0.1	6.7 ± 0.2
Partial portal vein ligated rats	10	43.1 ± 0.9	5.8 ± 0.3[a]	4.0 ± 0.2[a]	9.8 ± 0.5[a]
Cirrhotic rats	9	49.8 ± 0.9[a,b]	4.9 ± 0.1[a,b]	4.4 ± 0.2[a]	9.3 ± 0.3[a]

a: $p < 0.001$ in comparison to normal rats. b: $p < 0.05$ in comparison to partial portal vein ligated rats.

ed via a jejunal vein with a PE-50 catheter. The portal vein catheter was connected to a high sensitivity pressure transducer (Hewlett-Packard, model 1280 C, Waltham, Mass), calibrated before measurement. Permanent tracing of portal pressure was obtained with a multichannel recorder (Hewlett-Packard, 78309 A).

In order to avoid variations in blood volumes that might be due to differences in body weight between the study groups measurements of plasma volume, red blood cell volume and blood volume are expressed in ml/100 g bw.

Results

Portal pressure was increased to a similar extent in PPVL and cirrhotic rats (12.3 ± 0.4 and 12.7 ± 0.4 mm Hg, respectively) in relation to normal rats (6.0 ± 0.3 mm Hg) ($p < 0.001$).

The plasma volume and the calculated red blood cell volume and blood volumes were significantly higher in both groups of rats with portal hypertension than in the normal rats. Despite a similar red blood cell volume and blood volumes, cirrhotic rats had a higher hematocrit and a lesser increase in plasma volume than PPVL rats (table I).

Discussion

The present study demonstrates that blood volumes are markedly increased in rats with portal hypertension. Both the plasma volume and the calculated blood volume were significantly higher in partial portal vein ligated and in cirrhotic rats than in the control group of normal rats. Results in normal rats were similar to those of previously published studies (2, 24). The calculated blood volume was increased to a similar extent in partial portal vein ligated and cirrhotic rats (by 46% and 39% respectively) but the relative contribution of increases in plasma and red blood cell volumes were different in the partial portal vein ligated and in the cirrhotic rats. The plasma volume was increased by 45% in the former and by 23% in the latter group. Cirrhotic rats, because of a significantly higher hematocrit, had a higher increase in the calculated red blood cell volume (60%) than partial portal vein ligated rats (47%).

The reason for these increased blood volumes is not well known, but it probably represents an attempt to restore to normal the ratio of total blood volume to total vascular holding capacity, which is increased in portal hypertension (9). The mechanisms involved in the increased plasma volume are not well defined. Patients and animals with portal hipertension have been shown to present a hyperdynamic circulation evidenced by an increased cardiac output, decreased arterial pressure and total systemic resistance as well as an increased splanchnic blood flow (6, 12, 22, 23). These circulatory

abnormalities are thought to be important determinants of the changes in renal function and humoral vasoactive factors observed in portal hypertension (1). These changes may produce increased sodium reabsortion and impaired free water excretion (2, 9, 11), with a concomitant increase in plasma volume.

In conclusion, our results demonstrate that cirrhotic rats and partial portal vein ligated rats present a marked hypervolemia. Therefore, an increased plasma volume appears to be a universal finding in portal hypertension. These results should be considered in studies performed on these models of portal hypertension in the rat.

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Resumen

Se estudia la posible asociación de la hipertensión portal a modificaciones de la volemia, como se demuestra en otros modelos experimentales. El volumen plasmático se mide utilizando albúmina marcada con Tecnecio-99 en tres grupos de ratas macho Sprague-Dawley. Ratas normales utilizadas como grupo control, y dos grupos de ratas con hipertensión portal: uno con ligadura parcial de la vena porta y otro de ratas cirróticas mediante inhalación de Cl_4C. Las ratas con hipertensión portal presentan un aumento significativo del volumen plasmático en comparación con las ratas normales. El volumen sanguíneo es significativamente superior en los animales con hipertensión portal que en el grupo control. Se demuestra que la hipertensión portal en la rata está asociada a una marcada hipervolemia. Los resultados sugieren que los cambios en la volemia deben tomarse en consideración al efectuarse estudios hemodinámicos y farmacocinéticos en modelos experimentales en ratas con hipertensión portal.

Palabras clave: Hipertensión portal, Volemia.

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