The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl₄-cirrhotic liver

Alexander Zipprich,¹,² Mauricio R. Loureiro-Silva,¹,² Irita D'Silva,² and Roberto J. Groszmann¹,²

¹Digestive Disease Section, Yale University School of Medicine, New Haven, Connecticut; and ²Hepatic Hemodynamic Laboratory, Veterans Affairs Medical Center, West Haven, Connecticut

Submitted 30 April 2007; accepted in final form 15 May 2008

The liver has a dual blood supply through the portal vein and the hepatic artery. Portal venous blood flow corresponds to 70–80% and hepatic arterial blood flow to the hepatic artery. Portal venous blood flow is used to measure portal venous and sinusoidal pressures, as well as drug-induced decreases of elevated pressures. The aim of this study was to investigate the influence of hepatic arterial flow (HAF) changes on portal venous pressure (PVPP) and wedged hepatic venous pressure (WHVP). Normal and CCl₄-cirrhotic rats were subjected to a bivascular liver perfusion with continuous measurements of PVPP, WHVP, and hepatic arterial perfusion pressure. Flow-pressure curves were performed with the use of different flows either through the portal vein (PVF: 20–32 ml/min) or HAF (5–15 ml/min). Increases in HAF lead to significant absolute and relative increases in PVPP (P = 0.002) and WHVP (P < 0.001). Absolute changes in HAF correlated to absolute changes in PVPP (cirrhosis: r = 0.64, P < 0.001; control: r = 0.67, P < 0.001) and WHVP (cirrhosis: r = 0.71, P < 0.001; control: r = 0.82, P < 0.001). Changes in PVPP correlated to changes in WHVP due to changes in PVF only in cirrhosis (r = 0.75, P < 0.001), whereas changes in HAF correlated in both cirrhosis (r = 0.92, P < 0.001) and control (r = 0.77, P < 0.001). In conclusion, increases and decreases in HAF lead to respective changes in PVPP and WHVP. This suggests a direct influence of HAF on PVPP and WHVP most likely due to changes in sinusoidal perfusion.

The difference between wedged hepatic venous pressure and free hepatic venous pressure, are used to estimate elevated sinusoidal and portal venous pressures in patients with cirrhosis (12, 25). Most pharmacological therapies used to reduce portal pressure are based on the reduction of portal flow, and hepatic venous pressure gradient is used to monitor drug efficacy (2, 6, 9, 20). Indeed, it has been demonstrated that both hepatic venous pressure gradient and drug-induced reduction in hepatic venous pressure gradient are excellent predictors of survival and development of complications in patients with cirrhosis (2, 6, 9, 10, 20, 27, 28).

Data showing the influence of hepatic arterial blood flow on the hepatic venous pressure gradient in patients with cirrhosis are limited. In fact, these data are controversial, and the influence from hepatic arterial blood flow on the hepatic venous pressure gradient is difficult to conclude from these studies (23, 24). However, most of these studies compared baseline hepatic arterial blood flow and hepatic venous pressure gradient, whereas the influence of changes in hepatic arterial flow on hepatic venous pressure gradient has not been investigated. With the use of intravascular Doppler sonography it has been shown that adenosine-induced hepatic arterial vasodilation leads to an increase in hepatic venous pressure gradient (15). Although this was investigated only in a small number of patients with cirrhosis, it suggested a direct influence of changes in hepatic arterial flow on hepatic venous pressure gradient. However, the above-described concept of measuring drug efficacy by using hepatic venous pressure gradient assumes no significant contribution from the hepatic arterial flow to portal venous pressure and hepatic venous wedge pressure (16, 29, 30). Therefore, the aim of this study was to investigate the influence of different hepatic arterial and portal venous flows on wedged and portal pressure.

MATERIALS AND METHODS

Twenty-two control and cirrhotic male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) underwent sit liver perfusion. The American Physiological Society guide principles for the care and use of animals were followed. The Institutional Animal Care and Use Committee at the Veterans Affairs Media Center previously approved all procedures involving animals.

Induction of cirrhosis. Rats weighing 75–100 g underwent inhalation exposure of CCl₄ three times a week. Phenobarbital (0.35 g/l) was added to the drinking water as described previously (19). Treatment was given for ~14 wk. Perfusions were performed 6 to 10 days after the last doses of CCl₄ and phenobarbital. Age-matched rats were used as a control group.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
**In vivo measurement of portal pressure.** Rats were weighed and anesthetized with ketamine hydrochloride (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA; 100 mg/kg body wt) and diazepam (10 mg/kg body wt). Before each experiment, all pressure measurement systems were calibrated with the zero point at the level of the hepatic hilum. The abdomen was opened with a midline incision, and the ileocolic vein was cannulated. After a 10-min stabilization period, the in vivo portal venous pressure was measured. Portal hypertension was defined by a portal pressure higher than 10 mmHg (1).

**In situ rat liver perfusion.** After measurement of the portal pressure in vivo, xylazine (Rompum; Bayer, Shawnee Mission, KS; 40 mg/animal) was added, and an in situ bivascular liver perfusion via the portal vein and the hepatic artery was performed as previously described (7). Briefly, the opening of the abdomen was extended, and loose ligatures were placed around the aorta cranial of the celiac artery, around the mesenteric artery immediately after branching from the aorta, and the aorta caudal of the mesenteric artery. Left gastric and splenic artery were tied at its origin of the celiac artery. Left and right gastric arteries, as well as gastroduodenal arterial (branch of the common hepatic artery), were ligated. The bile duct was cannulated with a polyethylene tube (PE-10). Loose ligatures were placed around the inferior vena cava and the portal vein. The portal vein was cannulated with a 14-gauge Teflon catheter, and the ligature around the mesenteric artery was closed. The perfusion of the hepatic artery with 8 ml/min of oxygenated (carbon gas, 95% O₂-5% CO₂) Krebs-Henseleit solution containing dextrose (11 mM) in a nonrecirculating mode was started. The tip of the catheter was placed close to the branch of the hepatic artery, around the mesenteric artery immediately after branching from the aorta, and the aorta caudal of the mesenteric artery. Left gastric and splenic artery were tied at its origin of the celiac artery. Left and right gastric arteries, as well as gastroduodenal arterial (branch of the common hepatic artery), were ligated. The bile duct was cannulated with a polyethylene tube (PE-10). Loose ligatures were placed around the inferior vena cava and the portal vein. The portal vein was cannulated with a 14-gauge Teflon catheter, and the ligature around the mesenteric artery was closed. The perfusion of the hepatic artery with 8 ml/min of oxygenated (carbon gas, 95% O₂-5% CO₂) Krebs-Henseleit solution containing dextrose (11 mM) in a nonrecirculating mode was started. The tip of the catheter was placed close to the branch of the celiac artery, and all ligatures around the aorta were closed. A 14-gauge catheter was introduced in the inferior vena cava, and the thorax was opened.

To measure the sinusoidal pressure, a PE-60 catheter was guided from the right atrium, through the thoracic segment of the inferior vena cava into the left hepatic lobe, and wedged in the hepatic vein. The ligature around the superior vena cava was closed to secure the wedged catheter. The preparation was transferred to a temperature-controlled (37°C) Plexiglas perfusion chamber (Yale University Medical Instruments). The perfusion system was changed to a recirculating system, which allows us to use the wedged hepatic venous pressure as a continuous measurement and also as a proof of a wedged position during the entire experiment since a nonwedged position measurement would be closer to the zero reference point. Before each experiment, all pressure measurement systems were calibrated with the zero point at the level of the hepatic hilum. Perfusion and sinusoidal pressure were continuously recorded by Chart 3.6 program with the use of MacLab/4e hardware (AD instruments, Colorado Springs, CO). During the stabilization and experimental period, the perfusate was oxygenated with a Silastic tubing lung interposed between the perfusate reservoir and the peristaltic pump (13).

**Experimental design.** Normal and cirrhotic livers were perfused with constant flows during the stabilization period, and the flow through the wedged catheter was maintained. After the stabilization period, the wedged catheter outflow was interrupted to allow the measurement of the wedged hepatic venous pressure. During Experiment 1, the initial portal venous flow of 32 ml/min was reduced 2 ml/min every 2 min to a final flow of 20 ml/min. Next portal venous flow was reset to 32 ml/min, initiating a second 15-min period of stabilization. After this second stabilization period (Experiment 2), the hepatic arterial flow was first reduced from 8 ml/min to 5 ml/min and then increased to 10 and 15 ml/min with a 2-min interval between flows.

Portal venous vascular resistance and hepatic arterial vascular resistance were calculated by portal venous perfusion pressure and portal venous flow and by hepatic arterial perfusion pressure and hepatic arterial flow, respectively. Sinusoidal vascular resistance was calculated by wedge pressure and by total liver perfusion flow, i.e., the sum of portal venous and hepatic arterial flow.

Liver global viability was assessed by gross appearance of the liver, stable perfusion pressure, and bile production during the stabilization periods (>0.4 μl/min per g liver). After the experiment, liver and spleen were removed and weighed. Liver tissue samples were collected and fixed in formalin.

**Statistics.** Data are presented as means ± SE. Mann-Whitney U-test was used for comparisons of different groups at baseline level. Comparison for repeated measurements was assessed using the Friedman test to detect changes in each group (within group effects). Multivariate analysis of repeated measurements (ANOVA) was used to detect differences between control and cirrhotic groups (between group effect). The association between continuous variables was assessed with the Spearman rank correlation test. P values ≤ 0.05 were considered significant.

---

**Table 1. Absolute and relative changes in PVPP, HAPP, and WHVP due to changes in PVF (Experiment 1)**

<table>
<thead>
<tr>
<th>ΔPVF, ml/min</th>
<th>Animal Condition</th>
<th></th>
<th>ΔPVPP, mmHg (Δ%PVPP, %)</th>
<th>ΔHAPP, mmHg (Δ%HAPP, %)</th>
<th>ΔWHVP, mmHg (Δ%WHVP, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−2</td>
<td>Control</td>
<td></td>
<td>−0.22 ± 0.02 (−3.44 ± 0.34)</td>
<td>0.12 ± 0.10 (0.10 ± 0.10)</td>
<td>−0.08 ± 0.04 (−2.33 ± 1.34)</td>
</tr>
<tr>
<td>−5</td>
<td>Cirrhosis</td>
<td></td>
<td>−0.21 ± 0.06 (−3.28 ± 0.92)</td>
<td>−0.02 ± 0.70 (−0.02 ± 0.11)</td>
<td>−0.11 ± 0.04 (−2.88 ± 1.10)</td>
</tr>
<tr>
<td>−4</td>
<td>Control</td>
<td></td>
<td>−0.40 ± 0.04 (−6.21 ± 0.57)</td>
<td>0.32 ± 0.34 (0.25 ± 0.29)</td>
<td>−0.13 ± 0.07 (−3.55 ± 2.44)</td>
</tr>
<tr>
<td>−10</td>
<td>Cirrhosis</td>
<td></td>
<td>−0.45 ± 0.08 (−6.68 ± 1.29)</td>
<td>−0.15 ± 0.11 (−0.21 ± 0.16)</td>
<td>−0.21 ± 0.08 (−5.25 ± 2.14)</td>
</tr>
<tr>
<td>−6</td>
<td>Control</td>
<td></td>
<td>−0.63 ± 0.05 (−9.65 ± 0.67)</td>
<td>0.64 ± 0.54 (0.56 ± 0.45)</td>
<td>−0.19 ± 0.09 (−5.38 ± 3.11)</td>
</tr>
<tr>
<td>−15</td>
<td>Cirrhosis</td>
<td></td>
<td>−0.65 ± 0.11 (−9.81 ± 1.64)</td>
<td>−0.18 ± 0.16 (−0.27 ± 0.25)</td>
<td>−0.29 ± 0.16 (−7.17 ± 3.19)</td>
</tr>
<tr>
<td>−8</td>
<td>Control</td>
<td></td>
<td>−0.82 ± 0.05 (−12.63 ± 0.74)</td>
<td>0.89 ± 0.76 (0.79 ± 0.63)</td>
<td>−0.25 ± 0.10 (−7.17 ± 3.66)</td>
</tr>
<tr>
<td>−20</td>
<td>Cirrhosis</td>
<td></td>
<td>−0.85 ± 0.12 (−12.68 ± 1.68)</td>
<td>−0.18 ± 0.16 (−0.26 ± 0.24)</td>
<td>−0.37 ± 0.14 (−8.84 ± 3.92)</td>
</tr>
<tr>
<td>−10</td>
<td>Control</td>
<td></td>
<td>−1.02 ± 0.06 (−15.70 ± 0.95)</td>
<td>1.12 ± 0.91 (1.00 ± 0.76)</td>
<td>−0.29 ± 0.11 (−8.57 ± 3.64)</td>
</tr>
<tr>
<td>−25</td>
<td>Cirrhosis</td>
<td></td>
<td>−1.03 ± 0.15 (−15.27 ± 2.07)</td>
<td>−0.10 ± 0.14 (−0.15 ± 0.22)</td>
<td>−0.41 ± 0.17 (−9.81 ± 4.60)</td>
</tr>
<tr>
<td>−12</td>
<td>Control</td>
<td></td>
<td>−1.24 ± 0.07 (−19.10 ± 1.05)</td>
<td>1.22 ± 0.91 (1.15 ± 0.78)</td>
<td>−0.31 ± 0.12 (−9.28 ± 3.93)</td>
</tr>
<tr>
<td>−30</td>
<td>Control</td>
<td></td>
<td>−1.27 ± 0.15 (−18.83 ± 1.97)</td>
<td>0.003 ± 0.16 (0.02 ± 0.24)</td>
<td>−0.47 ± 0.19 (−11.20 ± 5.30)</td>
</tr>
</tbody>
</table>

Applicable values are means ± SE. Δ, Absolute change; Δ%, relative change; PVPP, portal venous perfusion pressure; HAPP, hepatic arterial perfusion pressure; WHVP, wedged hepatic venous pressure; PVF, portal vein flow; PVPP in control and cirrhosis conditions; P < 0.001 (within-group effect). WP: control, P = 0.002 (within-group effect); cirrhosis: P = 0.025 (within-group effect).
Changes of portal venous blood flow. The decrease of portal venous flow induced an increase of sinusoidal vascular resistance in cirrhotic \((P < 0.001)\) as well as control \((P < 0.001)\) animals (see Table 1). Furthermore, this decrease of portal venous flow correlated with the flow-induced increase of sinusoidal vascular resistance in cirrhotic \((r = -0.63, P < 0.001)\) as well as in control \((r = -0.52, P < 0.001)\) animals. However, this observed increase of sinusoidal vascular resistance was not significantly different between both groups. Decrease of portal venous flow did not lead to significant changes of hepatic arterial perfusion pressure in both groups.

Changes of portal venous vascular resistance. Decrease of portal venous flow caused significant changes of portal venous perfusion pressure and portal venous vascular resistance in cirrhotic \((P < 0.001)\) and control \((P < 0.001)\) animals but without significant differences between both groups.

Flow-induced changes of portal venous perfusion pressure correlated with changes of wedge pressure in cirrhotic animals \((r = 0.75, P < 0.001)\). Furthermore, changes of portal venous vascular resistance correlated well with changes of sinusoidal vascular resistance in cirrhotic animals \((r = 0.92, P < 0.001, \text{Fig. 1})\) and in control animals \((r = 0.67, P < 0.001)\).

Experiment 2

Changes of hepatic arterial perfusion pressure lead to significant changes of portal venous perfusion pressure and portal venous vascular resistance \((P < 0.01; \text{Fig. 2})\). The changes of hepatic arterial flow were, in addition, correlated with changes of portal venous perfusion pressure as well as portal venous vascular resistance in cirrhotic \((r = 0.64, P < 0.001)\) and in control \((r = 0.66, P < 0.001)\) animals (see Table 2). Interestingly, we also observed changes of wedge pressure due to changes in hepatic arterial flow. Changes in hepatic arterial flow caused changes of wedge pressure and sinusoidal vascular resistance in cirrhotic and control animals \((P < 0.01; \text{Fig. 2})\). Moreover, we found a correlation between changes of hepatic arterial flow and changes of wedge pressure in both groups \((\text{cirrhosis: } r = 0.71, P < 0.001; \text{control: } r = 0.82, P < 0.001)\). However, changes of portal venous perfusion pressure, portal venous vascular resistance, wedge pressure, and sinusoidal vascular resistance were not significantly different between cirrhotic and control animals due to changes of hepatic arterial flow (Fig. 2). Although there may be a trend to have different slopes between normal and cirrhotic livers, the slopes shown in Fig. 2 are not statistically different. Interestingly, the changes of portal venous perfusion pressure and wedge pressure in response to changes of hepatic arterial flow were highly correlated in both groups \((\text{cirrhosis: } r = 0.92, P < 0.001, \text{Fig. 3; control: } r = 0.77, P < 0.001)\).

Changes of hepatic arterial vascular resistance. In response to changes of hepatic arterial flow, cirrhotic animals had significantly smaller changes of hepatic arterial perfusion pressure \((15.7 \pm 0.8 \text{ vs. } 20.6 \pm 1.3 \text{ mmHg}; \text{ANOVA: } P = 0.01)\) and hepatic arterial vascular resistance \((-2.71 \pm 0.06 \text{ vs. } -3.15 \pm 0.30, P = 0.002)\).
The hepatic venous wedged pressure gives an excellent approximation to actual portal pressure and is used to monitor the effect of drug efficacy in portal hypertension (2, 12, 20).

Moreover, at the moment, it is the only method that can define the portal pressure response to pharmacological therapy since other clinical or radiological parameters do not reliably reflect this response (12). However, this concept of measuring drug efficacy using hepatic venous pressure gradient assumes that the decrease achieved on portal pressure and hepatic venous wedged pressure is all mediated by a decrease of portal venous inflow (16, 29, 30).

In the present study, we show a significant influence of hepatic arterial flow on portal venous and wedged pressure, as well as portal venous resistance and sinusoidal resistance in cirrhotic and normal animals. Moreover, we found a correlation between flow-induced changes of portal venous perfusion pressure and wedged pressure due to both changes in portal venous and hepatic arterial flow. Several studies have shown an excellent correlation between portal venous and hepatic venous wedged pressure in animals, as well as in humans (11, 12, 21).

In our study, portal venous perfusion pressure correlated well with the wedged pressure due to changes in portal venous and hepatic arterial flow. Animal studies investigating the influence of hepatic arterial flow on portal venous pressure found different results. Decreasing or stopping hepatic arterial flow modifies the portal venous pressure over a wide range (3, 8, 14, 22). Reduction in hepatic arterial flow causes decreases in portal venous pressure probably due to alterations in total blood flow through the sinusoids (3, 22). However, all of these studies were performed in normal animals and did not measure the sinusoidal resistance. Our study was performed in both cirrhotic and normal animals, and we measured the wedged pressure, a reflection of sinusoidal resistance. We found that changes of portal venous pressure and resistance in response to decreased and increased hepatic arterial flows were similar in cirrhotic and control animals. Our results with equal changes in sinusoidal resistance in both models support the hypothesis that alteration in total blood flow through the sinusoids is the main mechanism of change in portal resistance. Furthermore, the same response on wedged pressure and sinusoidal resistance following changes in flow either in the portal vein or in the hepatic artery support this hypothesis. Moreover, it indicates that, in the liver perfusion model, the portal venous vascular resistance is located in the sinusoids (3). On the other hand, changes in hepatic arterial flow were smaller in cirrhotic animals. Smaller increases in hepatic arterial flow caused equal increases in wedged pressure when we compared cirrhotic with control livers. This could be interpreted as a greater influence of hepatic arterial flow on wedged pressure in cirrhosis.
In cirrhotic animals, as well as in patients, hepatic arterial vascular resistance has been shown to be higher than, equal to, or lower than that without liver disease (5, 15, 23, 26, 30, 32, 33). We found a significantly smaller increase in hepatic arterial pressure in CCl₄-induced cirrhotic animals compared with normal animals in response to increase in hepatic arterial flow. The mechanisms involved in this vasodilatation have not been completely elucidated, but it has been shown that the hepatic artery is under local as well as systemic influence (15, 17). Therefore, an involvement of different locally and systemically produced vasodilatory factors like nitric oxide and adenine are possible (4, 32).

To investigate the influence of the hepatic arterial flow on portal venous and wedged pressure, we used a bivascular liver perfusion system. Although this preparation is established, there have been differences with in vivo measurements in recent years. The viscosity of the Krebs-Henseleit solution is lower than the viscosity of blood, which leads to a lower shear stress and subsequent to a lower perfusion pressure and vascular resistance. Furthermore, we could not observe a change of hepatic arterial vascular resistance due to changes of portal venous perfusion flow. Therefore, it seems that this preparation lacks the hepatic arterial buffer response. It was described by other investigators that the perfusion system does not show the normal hepatic arterial buffer response (3). However, in preliminary experiments (data not shown) with a greater decrease of portal venous flow, we found a marked decrease of hepatic arterial vascular resistance, showing the presence of the hepatic arterial buffer response in the used perfusion system.

In conclusion, this study demonstrates that changes in hepatic arterial flow lead to respective changes in portal venous and wedged pressure. Our findings indicating a direct influence of hepatic arterial flow on portal venous and wedged pressure most likely due to changes in total flow through the sinusoids. This was observed in cirrhotic and normal animals, and a similar reduction of portal venous and hepatic arterial flow lead to comparable reduction in wedged pressure.

**REFERENCES**


