Impact of intrinsic blood flow regulation in cirrhosis: maintenance of hepatic arterial buffer response

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The hepatic arterial buffer response (HABR) effectively controls total blood perfusion in normal livers, but little is known about blood flow regulation in cirrhosis. We therefore studied the impact of HABR on blood flow of cirrhotic livers in vivo. After 8-wk CCl4 treatment to induce cirrhosis, 18 anesthetized rats (and 18 non-cirrhotic controls) were used to simultaneously assess portal venous and hepatic arterial inflow with miniaturized ultrasonic flow probes. Stepwise hepatic arterial blood flow (HAF) or portal venous blood flow (PVF) reduction was performed. Cirrhotic livers revealed a significantly reduced total hepatic blood flow (12.3 ± 0.9 ml/min) due to markedly diminished PVF (7.3 ± 0.8 ml/min) but slightly increased HAF (5.0 ± 0.6 ml/min) compared with noncirrhotic controls (19.0 ± 1.6, 15.2 ± 1.3, and 3.8 ± 0.4 ml/min). PVF reduction caused a significant HABR, i.e., increase of HAF, in both normal and cirrhotic livers; however, buffer capacity of cirrhotic livers exceeded that of normal livers (P < 0.05) by 1.7- to 4.5-fold (PVF 80% and 20% of baseline). Persistent PVF reduction for 1, 2, and 6 h demonstrated constant HABR in both groups. Furthermore, HABR could be repetitively provoked, as analyzed by intermittent PVF reduction. HAF reduction did not induce changes of portal flow in either group. Because PVF is reduced in cirrhosis, the maintenance of HAF and the preserved HABR must be considered as a protective effect on overall hepatic circulation, counteracting impaired nutritive blood supply via the portal vein.

THE PATHOGENESIS OF CIRRHOSIS, which is initiated by hepatocyte necrosis and an inflammatory response with subsequent extracellular matrix deposition (8), leads finally to distinct alterations of the hepatic microvasculature (30). The cirrhosis-associated rarefaction of sinusoids (36, 37) and the structural changes of sinusoidal endothelia (12, 36) result in deteriorated nutritive blood supply of the liver, increased total hepatic vascular resistance, and, hence, portal hypertension and portosystemic collateralization (12, 31). Although major interest has been focused on rectifying blood flow disturbances (32), little is known about the function of regulatory mechanisms of hepatic blood flow in cirrhosis (11, 13, 27, 33).

Under physiological conditions, alterations of portal venous blood flow are counteracted by flow changes of the hepatic artery, aiming at the maintenance of total liver blood flow (14, 19). This regulatory mechanism, known as the hepatic arterial buffer response (HABR) and apparently regulated by adenosine (7, 15, 17, 24), serves not only to fulfill oxygen and metabolic demands of the liver (25) but also to control the overall metabolic well-being of the organism by maintaining hepatic clearance and excretory function (14, 15, 19). In hepatic cirrhosis, altered hemodynamics crucially deteriorate tissue oxygenation and liver function, and although the significance of hepatic arterial blood flow in various pathophysiological conditions has become evident (11, 20, 23, 34), the role of HABR in cirrhosis has not been extensively examined. Therefore, the aim of our study was to analyze the control of liver blood flow in cirrhotic rat livers with specific regard to the existence, persistence, and repeatability of HABR.

METHODS

Cirrhosis model. Experiments were performed in accordance with German legislation on protection of animals and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council).

Thirty-six Sprague-Dawley rats of either sex (body wt 301 ± 13 g; Charles River, Wiga, Sulzfeld, Germany) were divided into two groups. In group 1 (n = 18), animals were given phenobarbital sodium (35 mg/dl) in drinking water, and beginning 3 days later cirrhosis was induced by subcutaneous injection with 0.15 ml CCl4/100 g body wt (Merck, Darmstadt, Germany) in equal volumes of olive oil twice a week over a time period of 5 wk, as previously described (36, 37). Group 2 (n = 18) consisted of control animals receiving neither CCl4-olive oil injections nor phenobarbital sodium. All animals were kept on a standard dark-light cycle and were fed ad libitum with a stock pellet diet.

Surgical procedure. After overnight fasting with free access to tap water, animals were anesthetized with pentobarbital sodium (50 mg/kg body wt ip; Narcoren, Braun, Mel-
sungen, Germany), and supplemental doses (5 mg/kg body wt ip) were given during the experiment as required. Tracheotomy was performed to facilitate spontaneous breathing, and the animals were placed in a supine position on a heating pad maintaining body temperature at 36–37°C. Catheters (PE-50, 0.58-mm ID; Portex, Hythe, UK) were placed in the right carotid artery and jugular vein for continuous monitoring of mean arterial blood pressure (MAP) and for fluid substitution. After transverse laparotomy, microsurgical preparation for assessment of liver blood flow was performed similar to the method described by Lautt et al. (17) in cats. An ultrasonic perivascular flow probe (0.5 V; Transonic Systems, Ithaca, NY) was placed around the celiac artery, and all other branches including the splenic artery, the left gastric artery, and the gastroduodenal artery were ligated, so that all blood entering the hepatic artery was derived from the celiac artery. Likewise, a second flow probe (1.5 R; Transonic Systems) was positioned around the superior mesenteric artery, which, after ligation of all other inlet arteries to the splanchnic system (inferior mesenteric artery, anastomoses with rectal arteries), conducted blood flow solely representative of the portal vein. This experimental approach allowed simultaneous assessment of hepatic arterial and portal venous blood flow without the risk of mechanical obstruction or kinking of the referring vessels. An additional catheter (PE-50, 0.28-mm ID; Portex) was inserted via the splenic vein for continuous monitoring of portal venous blood pressure (PVP).

Blood flow measurements. After tourniquets (5-0 Ethibond; Ethicon, Norderstedt, Germany) were placed around the superior mesenteric and celiac arteries, stepwise reduction of blood flow of either of the feeding vessels to 80%, 60%, 40%, and 20% of baseline values was performed with a micromanipulator-controlled constrictor (cirrhosis, n = 6; controls, n = 6). Each individual step of portal venous flow

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Fig. 2. Hepatic arterial blood flow (●) in cirrhotic (A) and control (B) livers after reduction of portal venous blood flow (○) to 80% (II), 60% (III), 40% (IV), and 20% (V) of baseline (I). Values are means ± SE for triplicate measurements/animal (n = 6). §P < 0.05 vs. I, II, III, IV; *P < 0.05 vs. I, II, III; #P < 0.05 vs. I, II.

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Fig. 1. Histological section of liver tissue (Ladewig staining) after CCl₄ exposure for 8 wk. Note the dense fibrous septa dividing the hepatic parenchyma into multiple discrete nodules. Magnification, ×185.
Table 1. Hepatic arterial conductance and buffer capacity during consecutive reductions of portal venous blood flow

<table>
<thead>
<tr>
<th>Hepatic Arterial Conductance, ml/min⁻¹: kg·mmHg</th>
<th>Buffer Capacity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
<td>Controls</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.164 ± 0.021</td>
</tr>
<tr>
<td>80%</td>
<td>0.186 ± 0.024</td>
</tr>
<tr>
<td>60%</td>
<td>0.215 ± 0.027 †</td>
</tr>
<tr>
<td>40%</td>
<td>0.233 ± 0.028b</td>
</tr>
<tr>
<td>20%</td>
<td>0.247 ± 0.035b</td>
</tr>
</tbody>
</table>

Values are means ± SE. Percentages are percentage of baseline portal venous blood flow. †P < 0.05 vs. baseline; bP < 0.05 vs. baseline and 80%; cP < 0.05 vs. baseline, 80%, and 60%; dP < 0.05 vs. controls; eP < 0.05 vs. 80%.

Table 2. Hepatic arterial blood flow after 3 consecutive reductions of portal venous blood flow

<table>
<thead>
<tr>
<th>1st Reduction</th>
<th>2nd Reduction</th>
<th>3rd Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cirrhosis</td>
<td>Controls</td>
</tr>
<tr>
<td>Baseline</td>
<td>4.38 ± 0.57</td>
<td>4.15 ± 0.65</td>
</tr>
<tr>
<td>80%</td>
<td>4.47 ± 0.59</td>
<td>4.33 ± 0.53</td>
</tr>
<tr>
<td>60%</td>
<td>5.53 ± 0.57</td>
<td>5.12 ± 0.62</td>
</tr>
<tr>
<td>40%</td>
<td>6.34 ± 0.56</td>
<td>5.96 ± 0.79</td>
</tr>
<tr>
<td>20%</td>
<td>6.44 ± 0.92</td>
<td>7.21 ± 1.00</td>
</tr>
</tbody>
</table>

Values (in ml/min) are means ± SE. Comparison among 1st, 2nd, and 3rd measurements of hepatic arterial blood flow after portal venous flow reduction in cirrhotic livers (n = 6) and controls (n = 6) revealed no significant differences in either group.

Table 3. Coefficient of variance (relative dispersion) of percent changes of portal venous blood flow during consecutive reductions of portal venous blood flow

<table>
<thead>
<tr>
<th></th>
<th>Cirrhosis</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.069 ± 0.014</td>
<td>0.088 ± 0.031</td>
</tr>
<tr>
<td>80%</td>
<td>0.071 ± 0.004</td>
<td>0.088 ± 0.010</td>
</tr>
<tr>
<td>60%</td>
<td>0.110 ± 0.035</td>
<td>0.092 ± 0.021</td>
</tr>
<tr>
<td>40%</td>
<td>0.135 ± 0.036</td>
<td>0.129 ± 0.007</td>
</tr>
</tbody>
</table>

Values are means ± SE for triplicate measurements. Comparison within or between groups [cirrhotic livers (n = 6), controls (n = 6)] revealed no significant differences.

RESULTS

Rats treated with CCl₄ for 8 wk revealed a macroscopically nodular liver surface, a significant (P < 0.01) gain in liver weight (4.98 ± 0.13 g/100 g body wt vs. 3.22 ± 0.12 g/100 g body wt in controls), and histological signs of micronodular cirrhosis, i.e., dense fibrous septa dividing the hepatic parenchyma into multiple discrete nodules (Fig. 1). However, ascites and pronounced portosystemic collateralization were not evident.

In control animals, total liver blood flow showed values of 19.0 ± 1.6 ml/min with a portal venous blood flow of 15.2 ± 1.3 ml/min and a hepatic arterial flow of 3.8 ± 0.4 ml/min. In cirrhotic animals, total liver blood flow was found to be significantly reduced (12.3 ± 0.9 ml/min).

The ability of HABR was assessed by four consecutive cycles of portal venous flow reduction to 20% of baseline levels for 15 min with restoration of blood flow for another 15 min (n = 3 animals in each of the 2 groups).

Histopathology. Samples of liver tissue were fixed in 4% phosphate-buffered formalin for 2–3 days and embedded in paraffin. Sections (5 μm) were cut and mounted on poly-L-lysine slides for trichrome staining (Ladewig) to assess cirrhosis-associated collagen deposition.

Statistical analysis. All values are expressed as means ± SE. After the assumption of normality and homogeneity of variance across groups was proven, differences between groups were calculated using the unpaired Student's t-test (shown only in text to ensure clarity of figures). Differences between the individual occlusion steps within a group were assessed by one-way ANOVA (overall differences) followed by the Student-Newman-Keuls method (pairwise multiple comparisons). Overall statistical significance was set at P < 0.05. Statistics were performed using the software package SigmaStat (SPSS, Chicago, IL).
ml/min; \( P < 0.01 \) vs. controls) because of markedly diminished portal venous flow (7.3 \( \pm \) 0.8 ml/min; \( P < 0.01 \) vs. controls), whereas hepatic arterial flow increased (5.0 \( \pm \) 0.6 ml/min) comparably to that of non-cirrhotic controls. Thus the ratio of portal venous to hepatic arterial blood flow of 79.8% to 20.2% under control conditions changed to 59.3% to 40.7% in cirrhosis.

Stepwise reduction of portal venous blood flow initiated a pronounced and immediate HABR, i.e., an increase in hepatic arterial flow in both groups (Fig. 2). Concomitantly, hepatic arterial conductance increased progressively but without significant differences between the groups (Table 1). Repetition of the stepwise portal venous flow reduction did not influence the kinetics of the onset or the degree of HABR (Table 2). The degree of portal venous flow reduction did not vary markedly within the individual experiments or between the groups, as indicated by the quite low and almost unchanged coefficient of variance of percent change in portal venous blood flow (Table 3).

Flow reduction of the hepatic artery did not influence portal venous blood flow (Fig. 3). Repetition of stepwise flow reduction of hepatic arterial flow was also not associated with any significant change of portal venous blood flow in either of the two groups (data not shown). MAP of cirrhotic animals did not differ from that of noncirrhotic controls. Interestingly, reduction of portal venous blood flow to 20% resulted in a significant increase of MAP of \( \sim 10\% \)–15% in both groups (Fig. 4),

Fig. 3. Portal venous blood flow (•) in cirrhotic (A) and control (B) livers after reduction of hepatic arterial blood flow (○) to 80% (II), 60% (III), 40% (IV), and 20% (V) of baseline (I). Values are means ± SE for triplicate measurements/animal (n = 6).

Fig. 4. Mean arterial (○) and portal venous (•) blood pressure during portal venous blood flow reduction to 80% (II), 60% (III), 40% (IV), and 20% (V) of baseline (I) in cirrhotic (A) and control (B) livers. Values are means ± SE for triplicate measurements/animal (n = 6). *P < 0.05 vs. I, II, III, IV; §P < 0.05 vs. I, II; $P < 0.05 vs. I.
whereas reduction of hepatic arterial blood flow did not induce changes in MAP (Fig. 5). PVP was only slightly (although significantly, $P < 0.05$) higher in cirrhotic animals and remained almost unchanged by either hepatic arterial or portal venous flow reduction (Figs. 4 and 5).

Although portal venous flow reduction to 20% could not be thoroughly compensated for by HABR, the decrease of total liver blood flow was less pronounced in cirrhotic animals by $-2.1 \text{ ml/min} (-20.1\%)$ vs. $-8.5 \text{ ml/min} (-44.9\%)$ in controls; Fig. 6). Although the absolute value of HABR in cirrhosis is, on average, very similar to that in controls, the proportionate increase in hepatic arterial flow is strikingly augmented in cirrhosis. This is further reflected by the significantly higher buffer capacity in cirrhotic animals compared with controls (Table 1).

Portal venous flow reduction for 1, 2, or 6 h to 20% of baseline values demonstrated a constant HABR in both cirrhotic and control animals (Figs. 7 and 8). During the whole experimental time period, neither significant changes of blood flow (Figs. 7 and 8) nor changes in MAP and PVP (data not shown) occurred, thus indicating persistence of the intrinsic hepatic blood flow regulation over prolonged periods of compromised portal venous perfusion. Strikingly, reestablishment of portal venous perfusion resulted in immediate (<10 min) return of hepatic arterial blood flow, regardless of the duration of HABR (1, 2, or 6 h; Figs. 7 and 8).

Intermittent reduction of portal vein flow to 20% with intermediate restoration of baseline flow conditions showed that HABR could be repetitively provoked in both experimental groups (Fig. 9). Blood flow reduction was always accompanied by a slight decrease of PVP and an increase of MAP (Fig. 10). These changes were more pronounced than in stepwise flow reduction (Fig. 4), and fluctuation in MAP due to reduction and restoration of portal blood flow was signif-

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**Fig. 5.** Mean arterial (•) and portal venous (○) blood pressure during hepatic arterial blood flow reduction to 80% (II), 60% (III), 40% (IV), and 20% (V) of baseline (I) in cirrhotic (A) and control livers (B). Values are means ± SE for triplicate measurements/animal ($n = 6$).

**Fig. 6.** Total liver blood flow in cirrhotic (A) and control (B) livers with the contributing fractions of portal venous (open bars) and hepatic arterial (solid bars) blood flow under either baseline conditions (plain bars) or conditions of portal venous blood flow reduction to 20% (hatched bars). Values are means ± SE; $n = 6$. 

Fig. 7. Persistence of hepatic arterial buffer response (HABR), i.e., increase of hepatic arterial blood flow (●), in cirrhotic livers for 1 (A), 2 (B), and 6 (C) h of portal venous blood flow reduction to 20% (○). Values are means ± SE; n = 3 for each time period.

Fig. 8. Persistence of HABR, i.e., increase of hepatic arterial blood flow (●) in control livers for 1 (A), 2 (B), and 6 (C) h of portal venous blood flow reduction to 20% (○). Values are means ± SE; n = 3 for each time period.
DISCUSSION

A number of studies have been performed to analyze physiological control mechanisms of hepatic blood flow (2, 7, 14, 17, 19, 22, 26, 28). However, the significance of dual blood supply of the liver in various pathological states is still poorly understood (10, 16) or even neglected (4, 35). Although it is well known that major blood flow disturbances within the hepatic microcirculation occur in liver cirrhosis, only a few studies have addressed the role of hepatic arterial perfusion (5, 11, 13, 20, 23, 33).

In the present study, we demonstrated reduced total liver blood flow caused by reduced portal venous perfusion in cirrhosis while hepatic arterial blood flow was maintained. PVP was significantly elevated when compared with controls and fulfilled the criteria of portal hypertension (31). However, we did not observe excessive portal hypertension or manifest ascites as observed by others using different modes and models of cirrhosis induction (5, 6, 27, 32). The lack of excessive portal hypertension might be explained by the reduction or even elimination of hyperdynamic circulation on pentobarbital anesthesia (4, 21, 31). This view is further supported by our findings of normal MAP values in cirrhotic animals.

HABR has been examined in pigs (1, 9), dogs (11), sheep (34), and cats (7, 14, 15, 17, 19, 22). However, because one of the aims of our study was to set up a cirrhosis model in the rat that allows for measurement of hepatic blood flow, we adopted the technique described by Lautt et al. (17) with minor modifications.

Fig. 9. Repeatability of HABR, i.e., increase of hepatic arterial blood flow (●) during consecutive portal venous blood flow reduction to 20% (○) for 15 min and restoration of baseline flow conditions for 15 min in cirrhotic (A) and control (B) livers. Values are means ± SE; n = 3.

Fig. 10. Mean arterial (●) and portal venous (○) blood pressure during repetitive reduction of portal venous blood flow for 15 min and restoration to baseline flow conditions for 15 min in cirrhotic (A) and control (B) livers. Values are means ± SE; n = 3.
Although the use of perivascular flow probes has been regarded as technically difficult in the rat (29), transonic flowmeters have been proven to be accurate and highly reproducible (38). Therefore, the blood flow values obtained in the present study are consistent with those reported by others using flowmeters (38), microspheres (5, 21, 31), or clearance techniques (29).

The magnitude or efficiency of the buffer response varies widely depending on the technique used and the condition of the animal. In the context of variability of acute HABR under experimental conditions, Lautt (14) reported that HABR is generally greater at the early part of an experiment, and in some animals it became completely ineffective within 2 h of recording. Moreover, inasmuch as anesthesia might already have initiated some buffer response, we cannot exclude potential basal activation, although pentobarbital was reported not to influence HABR (19). In addition, to record hepatic arterial blood flow, the currently used methodology requires splenectomy, which would be expected to reduce portal flow and further activate the buffer response. The present methodology might underestimate the buffer capacity, but it allows us to evaluate the principle of hepatic artery responsiveness.

In addition to the classic concept of HABR, a reciprocal relationship between hepatic arterial and portal venous blood flow has also been proposed (3); this, however, was not observed in the present study. In addition, our results do not confirm the findings of Ayuse et al. (1) demonstrating that flow changes in the hepatic artery affect PVP.

Stepwise reduction of portal venous flow revealed a completely maintained HABR in cirrhosis, although it could be speculated that HABR had already been activated under these pathological conditions and might therefore be limited in extent. However, the enormous buffer capacity, with highest values between 50% and 60% in cirrhotic animals, indicates the maintenance of a remarkable potential of the hepatic artery to counteract reduced portal venous blood flow.

To our knowledge, no study has investigated the maintenance of HABR over prolonged time periods. We were able to demonstrate a constant HABR over a 6-h period of portal venous flow reduction in both normal and cirrhotic animals. These findings, together with the sustained repeatability of the buffer response, demonstrate the maintenance of HABR and underline the significance of this regulatory mechanism, particularly under the pathological conditions of cirrhosis.

Our results show that the degree of HABR in either cirrhotic or normal livers could not thoroughly compensate for diminished portal venous blood flow and maintain total liver blood flow. However, we show for the first time that the absolute reduction of total liver blood flow was less pronounced in cirrhotic than in control livers, because the proportionate increase of hepatic arterial flow is strikingly augmented in cirrhosis. Normally, the portal vein provides the major blood supply of oxygen to the liver (24). In cirrhosis, the change of the ratio of portal venous to hepatic arterial blood flow in favor of the hepatic artery may sustain oxygen delivery and exert a protective effect on organ function and integrity (25).

In conclusion, we established a reliable rat cirrhosis model that allows measurement of hepatic arterial and portal venous blood flow as well as mean arterial and portal venous blood pressure over experimental periods up to 6 h. Because portal venous blood flow is reduced in cirrhosis, the maintenance of hepatic arterial blood flow and the preserved HABR probably represent a beneficial mechanism for hepatic circulation, thereby counteracting impaired nutritive blood supply of the cirrhotic liver.

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