Hepatic Artery Flow and Propranolol Metabolism in Perfused Cirrhotic Rat Liver

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ABSTRACT
The oxygen limitation theory states that capillarization of the sinusoidal endothelium in cirrhosis impairs hepatocellular oxygen uptake manifesting as a reduction in oxygen-dependent enzyme activity including phase 1 drug metabolism. The hepatic artery supplies highly oxygenated blood to the liver. Therefore, we tested whether augmentation of hepatic arterial blood flow could improve hepatic oxygenation and function in cirrhosis. Rats were treated with carbon tetrachloride and phenobarbitone to induce hepatic cirrhosis or fibrosis. We used a bivascular rat liver perfusion model to examine the effects of increased hepatic artery flow on propranolol clearance and oxygen consumption. Each liver was perfused at three hepatic artery flow rates, 1 to 3, 4 to 6, and 7 to 9 ml/min with a constant portal venous flow of 7 to 9 ml/min. Increasing the hepatic artery flow led to improvement in propranolol clearance in control (n = 7, P < .001), fibrotic (n = 8, P < .001), and cirrhotic (n = 6, P < .001) livers. Intrinsic clearance of propranolol increased only in the cirrhotic livers (P = .01), indicating an improvement in enzyme activity. Regression analysis indicated that this improvement was mediated by change in oxygen delivery alone (P = .001). The results confirm that propranolol metabolizing enzyme activity in cirrhosis can be improved by increasing oxygen delivery by increasing hepatic arterial blood flow. These findings suggest that increasing hepatic arterial blood flow may be an important therapeutic strategy for improving global liver function in cirrhosis.

The determinants of impaired liver function, including drug metabolism in cirrhosis of the liver, are not fully elucidated (McLean and Morgan, 1991; Morgan and McLean, 1995). The traditional theories are 1) the sick cell theory, which assumes a global reduction in hepatocyte function; 2) the intact hepatocyte theory, which envisages a reduced mass of hepatocytes that function relatively normally and are normally perfused (Branch and Shand, 1976; Reichen et al., 1987); and 3) the impaired drug uptake theory, which assumes that drug elimination is reduced primarily because of impaired uptake of drug across the capillarized endothelium (Varin and Huet, 1985). Recently, the oxygen limitation theory was developed to explain the selective impairment of phase 1 drug metabolic pathways that accompanies cirrhosis of the liver and could not be accounted for by other theories (McLean and Morgan, 1991; Morgan and McLean, 1995). The central principle of this new theory is that phase 1 metabolism is more dependent than phase II metabolism on oxygen availability in the hepatocyte. Oxygen limitation in cirrhosis is thought to be secondary to capillarization (Popper et al., 1952) of the sinusoidal endothelium, which impedes the transfer of oxygen into the hepatocyte.

The central therapeutic implication of the oxygen limitation theory is that impaired liver function in cirrhosis should be overcome by increasing oxygen delivery to the liver. Oxygen supplementation did increase in vivo theophylline clearance in the cirrhotic rat (Hickey et al., 1995). Furthermore, in the perfused cirrhotic rat liver, increasing the oxygen concentration of portal venous perfusate improved propranolol clearance (Hickey et al., 1996). It has also been reported that through increasing portal venous flow to the perfused cirrhotic rat liver, which increased oxygen delivery, propranolol clearance was enhanced (Cardoso et al., 1994). Direct supplementation of inspired oxygen presents practical difficulties in humans, and increasing portal venous flow potentially could exacerbate portal hypertension. However, it may be possible to improve oxygenation of the cirrhotic liver by increasing hepatic artery flow to the liver, because the hepatic artery supplies about 20 to 33% of normal hepatic blood supply; importantly, this is highly oxygenated blood (pO2 approximately 100 mm Hg) (Lautt, 1976). The rest of the blood flow is via the portal vein, which, in contrast, delivers poorly oxygenated blood (pO2 approximately 40 mm Hg) (Lautt and Greenway, 1987). Accordingly, manipulation of the hepatic artery flow to the liver by selective oral vasodilators could provide an opportunity for improving liver function in cirrhosis. The feasibility of selective oral vasodilator delivery has been piloted in both dogs and humans (Heinzow et al., 1984; Gibson et al., 1987; Heinzow et al., 1987).

In this study, we have investigated whether increasing...
hepatic blood flow in the perfused livers of cirrhotic rats is associated with improved oxygen consumption and oxygen-dependent phase 1 drug metabolism. Propranolol was used as a marker of phase 1 metabolism because more than 90% of propranolol is metabolized by oxidation, and this metabolism is well characterized in normal and cirrhotic rat liver (Branch et al., 1973; Elliott et al., 1993; Fenvyes et al., 1993; Cardoso et al., 1994; Hickey et al., 1996).

Experimental Procedures

Materials. Propranolol, BSA, and taurocholic acid were obtained from Sigma Chemical Co. (Sydney, Australia), phenobarbital sodium was purchased from David Craig and Co. (Sydney, Australia), pentobarbital sodium was obtained from Boehringer Ingelheim Pty. Ltd. (Sydney, Australia), \( O_2/CO_2 \) and \( N_2/CO_2 \) gases were obtained from Linde Gas (Canberra, Australia), and acetonitrile and triethylamine were purchased from Ajax Chemicals (Sydney, Australia).

Induction of Cirrhosis. Male Wistar rats (80–100 g) were obtained from the John Curtin School of Medical Research, Australian National University (Canberra, Australia). Cirrhosis was induced by weekly gavage of carbon tetrachloride in corn oil for 8 to 10 weeks (5–50% \( \text{v/v} \)) and addition of phenobarbital sodium to drinking water commencing 2 weeks before CCl\(_4\) treatment (Proctor and Chatamra, 1982). Control rats had been treated with corn oil and phenobarbital sodium. The study was approved by the Australian National University Animal Experimentation Ethics Committee.

Bivascular Liver Perfusion. After anesthesia with pentobarbital sodium (60 mg/kg i.p.), laparatomy incision was made. The bile duct was cannulated with a 5-cm length of polyethylene tubing (i.d. 0.28 mm, o.d. 0.61 mm), the portal vein was cannulated with an 18-gauge i.v. cannula and the thoracic inferior vena cava with a 16-gauge i.v. catheter, and perfusion commenced. The hepatic artery was cannulated via the abdominal aorta with an 18-gauge i.v. cannula, and branches of the hepatic artery were ligated. The liver was perfused with Krebs-Henseleit buffer containing 20% out-of-date human erythrocytes (Red Cross Blood Bank, Canberra, Australia), perfused with Krebs-Henseleit buffer containing 20% out-of-date human erythrocytes (Red Cross Blood Bank, Canberra, Australia), and acetoni triate and triethylamine were purchased from Ajax Chemicals (Sydney, Australia).

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HPLC column using acetonitrile/water/triethylamine (33:69:1, pH 3.5) as mobile phase, and measured with a fluorescence detector (Schoeffel Instruments Corp., Sydney, Australia).

Data Analysis. The hepatic extraction ratio (\( E \)) was calculated from the inflow (\( C_{in} \)) and outflow (\( C_{out} \)) concentrations by the formula

\[
E = \frac{(C_{in} - C_{out})/C_{in}}{1} \quad (1)
\]

where \( C_{in} \) and \( C_{out} \) are the flow rates of the portal vein and hepatic artery, respectively.

The intrinsic clearance (\( CL \)) of propranolol, which is a model-dependent measure of total metabolizing enzyme activity, was determined from the extraction ratio with the parallel-tube model (Keiding and Steiness, 1984; Cardoso et al., 1994) according to the relationship

\[
CL = -\left(\frac{Q_{PV}}{Q_{HA}} + \frac{Q_{HA}}{Q_{PV}}\right) \cdot \ln(1-E)/f_u \quad (3)
\]

where \( f_u \) is the unbound fraction of propranolol.

Statistical Analyses. Data are presented as means ± S.D. Statistical significance was determined via linear regression analysis, ANOVA, and the two-tailed t test; differences were considered significant at \( P < .05 \). Forward stepwise progression was performed via Sigmastat version 2.0 (SPSS Inc., Chicago, IL).

Results

Induction of Cirrhosis. Hepatic cirrhosis was confirmed by histological examination in six of the treated rats and fibrosis in eight rats. The generation of both fibrotic and cirrhotic livers is a well-recognized feature of this type of treatment (Hall et al., 1991). The characteristics of the rats and their perfused livers are shown in Table 1. Bile flow, which has been reported to be either reduced (Hickey et al., 1996) or maintained (Krahenbuhl and Reichen, 1988) in cirrhosis, was significantly decreased in the cirrhotic livers.

Influence of Hepatic Artery Flow Rate on Propranolol Metabolism. There were no significant differences in flow rates (normalized for liver weight) and \( P_{O_2} \) values between the perfused livers of control, fibrotic, and cirrhotic rats, indicating that uniform perfusion conditions were achieved in all three groups (Table 2). There were no sequential changes in macroscopic appearance, portal venous resistance, or enzyme release during perfusions. Other parameters changed according to the changes in hepatic artery flow.

As expected, there were positive linear relationships between propranolol clearance and hepatic artery flow rate in the perfused livers of control (slope 1.2, \( P < .001 \)), fibrotic (slope 1.3, \( P < .001 \)), and cirrhotic (slope 1.0, \( P < .001 \)) rats (Fig. 1). The slope approximated unity in all three groups (Fig. 1). The slope approximated unity in all three groups. The clearance in cirrhotic livers was less than three-quarters of that observed in control livers.

A significant positive relationship between hepatic artery flow and calculated intrinsic clearance was limited to cirrhotic livers (\( P = .01 \)) (Fig. 2). Forward stepwise regression
There were no significant differences between groups. Values are means ± S.D.

### TABLE 1

In vivo parameters and initial measures of viability of perfused liver in control, fibrotic, and cirrhotic rats. Fibrotic and cirrhotic results are compared to control results. Values are means ± S.D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 7)</th>
<th>Fibrotic (n = 8)</th>
<th>P</th>
<th>Cirrhotic (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>494 ± 39</td>
<td>413 ± 40</td>
<td>.002</td>
<td>357 ± 68</td>
<td>.001</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>15.2 ± 3.1</td>
<td>15.8 ± 1.5</td>
<td>N.S.</td>
<td>11.3 ± 1.9</td>
<td>.02</td>
</tr>
<tr>
<td>Spleen weight (g)</td>
<td>1.7 ± 0.5</td>
<td>1.9 ± 0.4</td>
<td>N.S.</td>
<td>3.2 ± 0.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Plasma albumin (g/L)</td>
<td>27 ± 3</td>
<td>27 ± 3</td>
<td>N.S.</td>
<td>18 ± 1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Plasma alanine aminotransferase (U/L)</td>
<td>79 ± 25</td>
<td>85 ± 12</td>
<td>N.S.</td>
<td>115 ± 16</td>
<td>.02</td>
</tr>
<tr>
<td>Plasma aspartate aminotransferase (U/L)</td>
<td>134 ± 37</td>
<td>140 ± 33</td>
<td>N.S.</td>
<td>232 ± 60</td>
<td>.04</td>
</tr>
<tr>
<td>Plasma ALKP (U/L)</td>
<td>101 ± 22</td>
<td>126 ± 20</td>
<td>N.S.</td>
<td>321 ± 70</td>
<td>.007</td>
</tr>
<tr>
<td>Portal venous resistance (mm Hg · min⁻¹ · g⁻¹/ml)</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>N.S.</td>
<td>0.6 ± 0.3</td>
<td>.03</td>
</tr>
<tr>
<td>Hepatic arterial resistance (mm Hg · min⁻¹ · g⁻¹ · ml)</td>
<td>5.8 ± 1.9</td>
<td>4.9 ± 2.0</td>
<td>N.S.</td>
<td>3.9 ± 2.1</td>
<td>.005</td>
</tr>
<tr>
<td>Oxygen consumption (μmol · min⁻¹ · g⁻¹)</td>
<td>1.5 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>.03</td>
<td>1.0 ± 0.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Bile flow (mg/min)</td>
<td>12.8 ± 2.5</td>
<td>11.5 ± 4.0</td>
<td>N.S.</td>
<td>2.1 ± 1.2</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

N.S., not significant.

### TABLE 2

Initial perfusion conditions in control, fibrotic, and cirrhotic livers. There were no significant differences between groups. Values are means ± S.D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 7)</th>
<th>Fibrotic (n = 8)</th>
<th>Cirrhotic (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hepatic flow (ml · min⁻¹ · g⁻¹)</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Portal venous flow (ml · min⁻¹ · g⁻¹)</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Hepatic arterial flow (ml · min⁻¹ · g⁻¹)</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Portal venous pO₂ (mm Hg)</td>
<td>49 ± 8</td>
<td>43 ± 4</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>Hepatic arterial pO₂ (mm Hg)</td>
<td>119 ± 22</td>
<td>110 ± 20</td>
<td>108 ± 18</td>
</tr>
</tbody>
</table>

### Discussion

Apart from liver transplantation, few therapies are available to improve liver function in patients with cirrhosis of the liver. The oxygen limitation theory is important because it leads to the prediction that strategies to optimize liver oxygenation will improve liver function (Morgan and McLean, 1991). The hepatic arterial supply is an attractive target for such a therapeutic intervention because it carries highly oxygenated blood to the liver, and its flow rate potentially can be selectively influenced by oral vasodilators. The results of our study provide evidence to support the concept that manipulation of hepatic artery flow will improve liver function in cirrhosis.

Note that the perfused rat liver preparation used for these experiments is unusual. We used a bivascular preparation where the hepatic artery pO₂ was maintained at 100 mm Hg and the portal venous pO₂ at 40 mm Hg, which is similar to the preparation described by Gardemann et al. (1987). These partial pressures were chosen because they are similar to those observed in vivo and allow perturbations caused by biologically realistic changes in oxygen delivery to be assessed. Although these concentrations are physiological, they are much less than the 300- to 500-mm Hg concentrations often used in perfused liver preparations (Gores et al., 1986). Oxygen delivery was about 2 to 3 μmol · min⁻¹ · g⁻¹ in our preparation, which is similar to perfusions in which erythrocytes are omitted. The livers did not demonstrate any deterioration in viability parameters over the 60-min perfusion.
period. Furthermore, the propranolol clearances we measured are similar to those observed in normal and cirrhotic rat livers perfused with hyperoxygenated media (Branch et al., 1973; Elliott et al., 1993; Fenvyes et al., 1993; Cardoso et al., 1994; Hickey et al., 1996). Thus, this preparation is well suited for the purposes of our study.

We found that augmentation of hepatic artery flow rate to the cirrhotic liver was associated with an increase in the
model-dependent calculation of the intrinsic clearance of propranolol. The improvement in intrinsic clearance appeared to be mediated by increased oxygen delivery via the hepatic artery. Intrinsic clearance is a model-dependent measure of enzyme activity that, as we observed in normal livers (Fig. 2), would not be expected to be affected by blood flow (Keiding and Steiness, 1984). The results suggest that improved oxygenation of the cirrhotic liver had a direct effect on the activity of the phase 1 enzymes involved in propranolol metabolism, as predicted by the oxygen limitation theory (McLean and Morgan, 1991). Regardless of the possible mechanisms for our observations, increased hepatic arterial flow was clearly associated with improved propranolol clearance and activity of propranolol-metabolizing enzymes. We chose to examine propranolol because the metabolism of this compound is oxygen dependent (Hickey et al., 1996), but other oxygen-dependent metabolic functions will probably be optimized by increasing hepatic artery blood flow. Therefore, strategies to improve hepatic arterial flow may be useful therapeutically. Note particularly that vasoactive agents given via the portal vein can influence hepatic artery flow (Richardson and Withrington, 1981). Vasodilators that are highly extracted by the liver and highly selective for the hepatic arterioles may have a selective effect on hepatic artery hemodynamics when given orally and in low dosage. Such agents might include Ca$^{2+}$ channel blockers, nitrates, and some β-blockers (Phillips et al., 1998). Delivery of this type of therapy must avoid significant systemic hypotension to avoid secondary reduction in hepatic arterial flow by reducing aortic pressures.

Various mechanistic linkages could exist between hepatic artery flow change and improved liver function in cirrhosis. Propranolol delivered via the hepatic artery may be preferentially metabolized in cirrhosis. The observations that the metabolism of lignocaine, meperidine (Ahmad et al., 1984), and phenacetin (Pang et al., 1994) are less efficient when administered into the hepatic artery make this hypothesis unlikely. Increased hepatic arterial flow could increase recruitment of sinusoids in cirrhosis; however, we simultaneously perfused the livers via the portal vein at flow rates above 10 ml/min, a rate sufficient to prevent baseline derecruitment (Pang et al., 1988). An alternate explanation is metabolic oxygen steal. Oxygen might preferentially be used by other cellular processes in cirrhosis, such as inflammation and regeneration, rather than drug metabolism. However, this is not supported by the results shown in Fig. 4, where the slope of the relationship between blood flow and oxygen consumption is less steep in cirrhotic than in control livers. The next oxygen-based explanation relates to a rate-limiting oxygen deficit confined to cirrhosis—the oxygen limitation theory. The finding that intrinsic clearance of propranolol in the cirrhotic liver was influenced directly by the delivery of oxygen via the hepatic artery supports this theory. Note that the oxygen limitation theory is not at variance with the intact hepatocyte theory, because it assumes there is impaired oxygen delivery to potentially normal cells. Finally, it has been hypothesized that propranolol metabolism is impaired in cirrhosis because of a barrier to propranolol uptake posed by the capillarized sinusoidal endothelium (Fenvyes et al., 1993; Gariepy et al., 1993). Hepatocellular uptake of substrates can be improved by increasing sinusoidal volume (Le Couteur et al., 1995), which might occur at higher hepatic artery flow rates in cirrhotic livers and compensate for any uptake barrier. Our data are also consistent with this explanation.

In summary, we found that augmentation of hepatic artery flow within the physiological range caused an increase in propranolol-activity. This was associated with increased hepatic oxygenation and supports the oxygen limitation theory of cirrhosis but is also consistent with other theories. The most important conclusion suggested by the results is that selective increase in hepatic arterial blood flow may be an important therapeutic strategy in the management of hepatic cirrhosis.

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References


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