Influence of acidosis and hypoxia on liver ischemia and reperfusion injury in an in vivo rat model

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group, rats were endotracheally intubated (14-gauge Venflon) and ventilated (Zoovent ventilator, Instrument, Amerongen, The Netherlands) with a mixture of oxygen and air (1:1 vol/vol, 2 l/min) without the use of positive end-expiratory pressure. Adequate ventilation was confirmed by continuous monitoring of end-tidal CO₂. In this group, we aimed at maintaining a physiological pH without development of hypoxia. In the second group, rats received oxygen and air (1:2 vol/vol, 3 l/min) via a mask while they were breathing spontaneously without further respiratory support. These rats should develop acidosis but not hypoxia. In the bicarbonate-treated group, rats received bicarbonate infusion (2.1%, 3 ml/h) via the tail vein while respiratory support was not provided. These rats should develop hypoxia without acidosis. In the control group, rats were breathing spontaneously without any form of respiratory or metabolic support. These rats will develop acidosis and hypoxia.

Surgical procedure. In all animals, a silicone catheter (diameter 0.9 mm) was inserted into the right carotid artery for assessment of hemodynamic parameters during surgery and for collection of blood samples. After midline laparotomy, the common bile duct was cannulated with a polyethylene catheter (diameter 0.4 mm) for collection of bile. Bile was continuously collected before ischemia (15 min), during ischemia (60 min), and during reperfusion (90 min) as a parameter of hepatocyte function. After dissection of the falciform ligament, the afferent vessels to the median and left lateral lobes were exposed by elevating the hepatic lobes upward. An atraumatic vascular clip was applied to these vessels to induce partial hepatic ischemia (70%) during 60 min. At the end of the ischemic period, the clip was removed, and subsequently without further respiratory support. These rats should develop hypoxia without acidosis. In the control group, rats were breathing spontaneously without any form of respiratory or metabolic support. These rats will develop acidosis and hypoxia.

Blood sampling. Blood samples (500 µl) were collected before induction of ischemia, at the end of the ischemic period, and after 90 min of reperfusion in Microtainer tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged (10 min, 3,000 rpm, 4°C), and plasma was collected for the assessment of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as parameters for hepatocellular injury. Activities of AST and ALT in plasma were determined by routine laboratory testing. Preischemia, at the end of the ischemic period, and after 90 min of reperfusion, routine arterial blood-gas analysis was performed (ABL 505 and OSM 3 hemoximeter, Radiometer, Copenhagen, Denmark).

For calculation of mean and significant differences, pH values were converted to blood hydrogen ion concentrations and expressed as means ± SE. However, for clarity, mean pH values are also mentioned in Table 1.

Table 1. Mean arterial blood pressure and arterial pH, PÇO₂, and PO₂ values measured before induction of partial liver ischemia, at the end of 60 min of ischemia, and after 90 min of reperfusion

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Ventilated</th>
<th>Mask</th>
<th>Bicarbonate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-I</td>
<td>67 ± 2</td>
<td>68 ± 1</td>
<td>66 ± 2</td>
<td>65 ± 4*</td>
</tr>
<tr>
<td>End-I</td>
<td>62 ± 2*</td>
<td>67 ± 2*</td>
<td>64 ± 2*</td>
<td>56 ± 2*</td>
</tr>
<tr>
<td>90 min R</td>
<td>66 ± 2*</td>
<td>68 ± 2*</td>
<td>64 ± 2*</td>
<td>55 ± 4*</td>
</tr>
<tr>
<td>H⁺, nM (pH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-I</td>
<td>38.9 ± 1.7(7.41)</td>
<td>46.9 ± 4.7* (7.33)</td>
<td>39.0 ± 1.3(7.41)</td>
<td>43.2 ± 1.9*</td>
</tr>
<tr>
<td>End-I</td>
<td>38.8 ± 1.7*d (7.41)</td>
<td>64.2 ± 4.7*h,b,e,i,j,m (7.19)</td>
<td>39.3 ± 2.5<em>h,</em> (7.41)</td>
<td>53.9 ± 1.5</td>
</tr>
<tr>
<td>90 min R</td>
<td>38.2 ± 1.1h,k (7.42)</td>
<td>62.5 ± 3.2h,i,j,m (7.20)</td>
<td>36.1 ± 1.2* (7.44)</td>
<td>55.7 ± 1.9</td>
</tr>
<tr>
<td>PÇO₂, Torr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-I</td>
<td>35.6 ± 1.4*</td>
<td>49.3 ± 5.1*</td>
<td>44.7 ± 2.1*</td>
<td>43.7 ± 2.9</td>
</tr>
<tr>
<td>End-I</td>
<td>32.1 ± 1.9h,e,d</td>
<td>64.8 ± 4.2*e,f</td>
<td>49.6 ± 3.5*</td>
<td>52.4 ± 4.0*</td>
</tr>
<tr>
<td>90 min R</td>
<td>31.6 ± 1.3h,b,i,k,l</td>
<td>62.4 ± 1.9*</td>
<td>52.1 ± 1.5*j,m</td>
<td>51.2 ± 2.1</td>
</tr>
<tr>
<td>Bicarbonate, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-I</td>
<td>22.1 ± 0.4*h,b,e</td>
<td>25.2 ± 0.7*d</td>
<td>27.6 ± 0.7h,d,e</td>
<td>24.6 ± 0.9*</td>
</tr>
<tr>
<td>End-I</td>
<td>20.2 ± 0.4 *e,h,g</td>
<td>23.7 ± 0.4*i</td>
<td>30.6 ± 1.0*</td>
<td>22.8 ± 0.5*</td>
</tr>
<tr>
<td>90 min R</td>
<td>20.1 ± 0.8*</td>
<td>23.3 ± 0.9*m,n</td>
<td>35.2 ± 1.0</td>
<td>22.9 ± 0.6*</td>
</tr>
<tr>
<td>PO₂, Torr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-I</td>
<td>221 ± 11*h</td>
<td>195 ± 17*d</td>
<td>87 ± 7*e,c,p</td>
<td>78 ± 11</td>
</tr>
<tr>
<td>End-I</td>
<td>195 ± 11*</td>
<td>133 ± 17*h,b,i</td>
<td>63 ± 3f,h,j</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>90 min R</td>
<td>197 ± 3n,m</td>
<td>127 ± 14</td>
<td>49 ± 2*m,p</td>
<td>49 ± 3n-q</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial blood pressure; pre-I, before induction of partial liver ischemia; end-I, at the end of 60 min of ischemia; 90 min R, after 90 min of reperfusion. Control rats showed a decrease in MAP at the end of ischemia and after 90 min of reperfusion. Physiological pH was maintained in ventilated and bicarbonate-treated rats, whereas mask and control rats developed acidosis (see MATERIALS and METHODS). An accumulation of CO₂ was observed in all rats, except for ventilated rats. Bicarbonate levels were different between groups. All rats showed a stable bicarbonate concentration during the experiment, except bicarbonate-treated rats. PO₂ values in bicarbonate-treated and control rats were significantly lower than in ventilated rats and rats with an oxygen mask. Significant differences exist between values with identical superscript letters, P < 0.05.
were performed using SPSS version 10.1 for Windows. A value of $P < 0.05$ was considered significant.

RESULTS

**Hemodynamics.** Mean arterial blood pressure (MAP) returned to preischemic values within 5–10 min after vascular inflow occlusion, and splanchnic congestion was not observed because of partial occlusion of the afferent liver vessels. Animals in all groups, except for the nonsupported control group, showed stable hemodynamics during the entire liver I/R procedure. At the end of the ischemic period and after 90 min of reperfusion, rats in the control group showed a lower MAP compared with all other groups. After 90 min of reperfusion, MAP in the control group was significantly decreased compared with the preischemic values (Table 1).

**pH.** Ventilated rats and rats in the bicarbonate-treated group showed a physiological blood pH during the entire procedure. Rats in the control group developed acidosis during the ischemic period, with pH values lower than those in ventilated rats and bicarbonate-treated rats. Rats with an oxygen mask showed the lowest pH (Table 1).

**Bicarbonate.** Blood bicarbonate levels were lowest in ventilated rats and highest in rats treated with bicarbonate. Bicarbonate levels remained stable in all rats during the experiment, except in bicarbonate-treated rats (Table 1).

**$PCO_2$.** All nonventilated rats showed increased blood $PCO_2$ levels, whereas normal blood $PCO_2$ levels were maintained in the ventilated rats. Blood $PCO_2$ levels were highest in rats in the mask group, followed by $PCO_2$ levels in rats in the control group and the bicarbonate-treated group (Table 1).

**$PO_2$.** Although rats in the mask group showed lower blood $PO_2$ after ischemia compared with ventilated rats, blood $PO_2$ values in both groups were sufficient to maintain adequate organ oxygen supply during the experiment. In contrast, rats in both the bicarbonate-treated and control groups showed low $PO_2$ immediately after induction of anesthesia. After ischemia, $PO_2$ dropped to critically low levels in both the bicarbonate-treated and the control groups (Table 1).

**Hepatocellular injury and function.** AST and ALT levels at the end of ischemia and after 90 min of reperfusion showed significant elevation in rats of all groups compared with preischemic values (Fig. 1). After 90 min of reperfusion, plasma AST and ALT levels were lower in the ventilated rats compared with all other groups. AST and ALT levels of rats in the bicarbonate-treated group were higher compared with rats in the mask and control groups.

**Overall, bile secretion was significantly reduced during liver ischemia and during 90-min reperfusion compared with preischemic levels in all rats. None of the animals showed a significant recovery of bile secretion after 90 min of reperfusion. Furthermore, bile secretion was not significantly different between rats in all groups (Fig. 2).**

**Histopathology.** In hematoxylin- and eosin-stained paraffin sections of the I/R liver lobes illustrated in

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**Fig. 1.** Plasma aspartate aminotransferase (AST; A) and alanine aminotransferase (ALT; B) measured before induction of partial liver ischemia (pre-I), at the end of 60 min of ischemia (end-I), and after 90 min of reperfusion (90 min R) in ventilated rats (hatched bars), rats with an oxygen mask (gray bars), bicarbonate-treated rats (open bars), and control rats (solid bars). AST and ALT levels were lowest in ventilated rats and highest in bicarbonate-treated rats. Values are means ± SE. *Significantly different from ventilated rats, $P < 0.05$. †Significantly different from bicarbonate-treated rats, $P < 0.05$.

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**Fig. 2.** Bile secretion measured during 15 min before induction of partial ischemia (pre-I), during ischemia (during I), and during 90 min of reperfusion (90 min R) in ventilated rats (hatched bars), rats with an oxygen mask (gray bars), bicarbonate-treated rats (open bars), and control rats (solid bars). Values are means ± SE. All groups showed significant reduction in bile secretion during ischemia and reperfusion compared with preischemic bile secretion (significance not shown).
Fig. 3, the semiquantitative scoring of liver injury corresponded to the degree of parenchymal injury indicated by the plasma AST and ALT levels found in all groups (mean scores: ventilated rats 2.5, mask group 3.0, bicarbonate-treated group 4.2, and control group 3.2), whereas the non-I/R control lobes showed none of the investigated alterations (see MATERIALS AND METHODS).

**DISCUSSION**

The results of this study emphasize that the development of acidosis and/or hypoxia during in vivo liver I/R experiments has substantial impact on the extent of liver I/R injury. Temporary cross-clamping of the pedicle to the left lateral and median lobes of the liver is a frequently applied in vivo model for partial (70%) liver I/R in the rat. The absence of severe splanchnic congestion is a major advantage of this model, because splanchnic congestion, when prolonged for >20 min, will result in circulatory shock with intestinal ischemia, unless a concomitant decompressing portal venous-systemic shunt is created (6). Because venous return to the heart is not compromised in this model, only minor temporary changes in blood pressure were observed directly after selective vascular inflow occlusion.

A review of the literature on the use of the partial liver ischemia rat model revealed that anesthesia appears to be induced and maintained with numerous drugs (chloral hydrate, pentobarbital, atropine, ketamine, xylazine, diazepam, Hypnorm, urethane, isoflurane, halothane, metofane, ether, and multiple combinations). Drugs are administered via intramuscular and intraperitoneal injections as well as by inhalation and by ventilation. Our findings suggest that comparison among published results obtained by using a 70% partial liver I/R rat model should take into account the method of anesthesia applied and, accordingly, should be made with caution.

In this study, nonventilated rats with bicarbonate infusion retained a physiological pH, whereas other nonventilated rats developed acidosis. Also, nonventilated rats developed hypoxia when oxygen was not supplied. Ventilated rats maintained normocapnia, a physiological pH, and mild hyperoxia. Hypercapnia and hypoxia in the nonventilated rats are evidently caused by inadequate gas exchange in the alveoli because of inadequate respiration, leading to respiratory acidosis. At the end of the ischemic period, when the hepatic circulation was not yet restored, liver-derived lactic acid unlikely played a role in the development of acidosis. Moreover, differences in pH between rats with an oxygen mask and ventilated rats already existed before induction of ischemia. During reperfusion, pH remained stable in all groups (as did the PCO2 and bicarbonate levels), which makes the contribution of lactic acid to the prolongation of acidosis less likely.

The upper Po2 limit in the ventilated rats was 200 Torr, which is higher than normal Po2 (~94 Torr). It has been reported that hyperoxia (induced by exposure to 100% oxygen under normobaric conditions) can increase reactive oxygen species formation in different organs after at least 24 h of exposure (1, 12, 13). Because in our study ventilated rats were exposed to high oxygen pressures for a maximum of 3 h, hyperoxia is not thought to contribute to the development of liver I/R injury.

Although rats with an oxygen mask showed increased respiratory insufficiency (i.e., increased PCO2 levels) and significantly lower blood Po2 after ischemia compared with ventilated rats, Po2 values were still above physiological levels. This suggests that the differences in arterial Po2 unlikely contributed to the differences in liver I/R injury between rats with an oxygen mask and ventilated rats.

Rats with an oxygen mask showed significantly enhanced hepatocellular I/R injury compared with ventilated rats. This suggests that hypercarbic acidosis during normoxic reperfusion has an adverse effect on liver I/R injury. In our experiments, PCO2 levels did not exceed 65 Torr, and, therefore, only mild acidosis developed. Although it has been reported that mild acidosis can be protective, whereas severe acidosis can enhance hypoxic injury in isolated hepatocytes (7), the present experiments show opposing results under normoxic conditions. Our data suggest that enhanced hepatocellular I/R injury, despite normoxic reperfusion in rats with an oxygen mask, was due to respiratory acidosis.

In contrast, rats of the control group with mild acidosis and severe hypoxia during liver I/R showed significantly less hepatocellular injury compared with bicarbonate-treated rats with hypoxia only. The protective effect of acidosis under hypoxic conditions is well documented in the literature (2, 4). Some studies suggest that the supply of bicarbonate may well restore blood pH values but that it leads to a paradoxical decrease of intracellular pH and hence to impaired cellular function (9, 10, 17). This assumption is still highly controversial, and other studies suggest that the
role of this phenomenon is minimal (5, 11, 14) and transient (15) in vivo.

The critically low $P_{O_2}$ during liver I/R in nonventilated control and bicarbonate-treated rats actually prolonged the hypoxic period of the liver during reperfusion, despite restoration of blood flow. Reportedly, prolonged hypoxia during reperfusion severely augments liver I/R injury (16). As was mentioned before, mild acidosis in hypoxic, nonventilated control rats attenuated liver I/R injury during hypoxia compared with that in hypoxic, nonventilated bicarbonate-treated rats with physiological pH. Mild acidosis did not completely protect the liver from the adverse effects of hypoxic reperfusion, because plasma AST and ALT levels in nonventilated, hypoxic control rats with physiological pH were still significantly higher compared with those in ventilated, normoxic rats with physiological pH.

Bile secretion is considered to be a good indicator of hepatocyte function (3). However, in this study during the entire period of liver I/R, bile secretion was not significantly altered by acidosis and/or hypoxia, despite large differences in hepatocellular I/R injury. Although prolonged observation may reveal larger differences among the groups, previous studies in our laboratory showed incomplete recovery of bile secretion also after 24 h of reperfusion (data not shown).

In conclusion, the results of this study suggest that respiratory acidosis significantly enhanced liver I/R injury under normoxic conditions, whereas respiratory acidosis significantly reduced liver I/R injury under hypoxic conditions. Clearly, standardization of methods used in I/R studies is crucial for comparison and validation of animal models. This should include, as is our conclusion from the data presented here, controlled respiratory support of the animal during ischemia and reperfusion of the liver.

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REFERENCES


