Protective Effects of Polyethylene Glycol (20 mol/L) in Phosphate-Buffered Sucrose for Rat Liver Preservation

I. Ahmed, N. Ahmad, M.S. Attia, J.P.A. Lodge, and D.J. Potts

The use of polyethylene glycol (PEG) in preservation solutions has been associated with a decreased incidence of rejection in clinical and experimental transplantation. Using the isolated perfused rat liver model, we found that PEG, when added to phosphate-buffered sucrose solution (PBSL), provided improved liver preservation. A direct comparison of three different molecular configurations of PEG in phosphate-buffered sucrose (PBSL) was carried out.

The following solutions were studied in our isolated perfused rat liver (IPRL) model:

- PBSL-I (without PEG);
- PBSL-II (containing polyethylene glycol; MW 3350);
- PBSL-III (containing polyethylene glycol; MW 8000); and
- PBSL-IV (containing polyethylene glycol; MW 20,000).

MATERIALS AND METHODS

The experiment was carried under a home office license according to standard guidelines for animal care. Male Wistar rats, weighing 280 to 300 g, were obtained from Biomedical Services, University of Leeds. Rats were anesthetized by a single intraperitoneal injection of pentobarbital (0.6 µg/g of body weight) without prior fasting. The abdomen was opened by a longitudinal midline and transverse subcostal incision. The common bile duct was visualized and cannulated with a fine cannula of 0.28-mm diameter. The cannula was secured in position with a cotton thread. The bile duct was divided at the distal end after cannulation.

Heparin (1000 U) was then injected via the penile vein. The abdominal aorta was isolated and cannulated with a 2-mm-diameter polyethylene catheter, which was held in position by a bulldog clamp. The thoracic cavity was opened and the supradiaphragmatic aorta clamped. The inferior vena cava was cut to allow the experimental fluid to escape freely into the chest cavity after perfusion of the liver. Thirty milliliters of ice-cold experimental solution was run through the aorta to perfuse the liver via the hepatic portal system.

Table 1. Weight Change (% Age)

<table>
<thead>
<tr>
<th>Solution</th>
<th>After Preservation (g)</th>
<th>After Reperfusion (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBSL-I</td>
<td>3.976 ± 0.9843*</td>
<td>7.348 ± 1.332*</td>
</tr>
<tr>
<td>PBSL-II</td>
<td>2.684 ± 1.2566*</td>
<td>4.095 ± 1.4421*</td>
</tr>
<tr>
<td>PBSL-III</td>
<td>0.3744 ± 0.9321</td>
<td>3.442 ± 1.2521*</td>
</tr>
<tr>
<td>PBSL-IV</td>
<td>2.973 ± 0.1211</td>
<td>0.0925 ± 1.4312</td>
</tr>
</tbody>
</table>

Data expressed as Mean ± SEM.

* P < .05 (one-way analysis of variance).

From the Department of Organ Transplantation, St James University Hospital, Leeds, UK (I.A., N.A., M.S.A., J.P.A.L.); and School of Biomedical Sciences, University of Leeds, Leeds, UK (D.J.P.).

Supported by the Royal College of Surgeons of England and Trustees of St James University Hospital, Leeds, UK (combined transplant funds).

Address reprint requests to Dr David J. Potts, School of Biomedical Sciences, Worsley Building, Level 10, University of Leeds, Leeds LS2 9NQ, UK. E-mail: d.j.potts@leeds.ac.uk.

© 2001 by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010
hepatic artery and allowed to escape through the hepatic veins and interior vena cava (IVC) into the chest cavity.

The portal vein was then visualized. A 2-mm polyethylene cannula was inserted and secured with a cotton tie. Another 20 mL of ice-cold preservative solution was allowed to run through the portal vein. The cannula was then capped while making sure no air bubbles entered.

The liver was mobilized, removed, and stored in 60 mL of experimental solution at 4°C for 24 hours.

Following storage, the liver was perfused at 37°C with physiologic saline containing 5% bovine serum albumin to maintain oncotic pressure, and 40% bovine red cells to carry oxygen to the liver.

Liver function was observed by means of a conventional isolated perfused liver system within a 37°C cabinet. The liver was perfused with MOPS saline containing 5% bovine serum albumin to maintain oncotic pressure and 40% bovine red cells to provide good oxygenation.

The perfusate was drawn at a constant flow rate of 15 mL/min from the reservoir positioned on a magnetic stirrer by a roller peristaltic pump. It was equilibrated with 95% oxygen and 5% carbon dioxide in the oxygenator, passed through a debubbler, and perfused through the liver. It was allowed to escape from the cut vena cava from which it was returned to the reservoir. Perfusion pressure was monitored continuously at the portal vein catheter. A sampling port positioned immediately prior to the portal vein cannula facilitated collection of portal vein samples; samples were also obtained from the emerging fluid. Perfusion continued for 2 hours.

Samples were collected from the portal vein and hepatic vein after every 30 minutes and checked for pH, partial pressure of oxygen, and carbon dioxide.

RESULTS

The following parameters were recorded and/or calculated:
1. Weight change after preservation and after reperfusion.
2. Bile flow.
3. Liver enzymes, including AST, ALT, and LDH.
4. Oxygen consumption.
5. Portal vein pressure.

PBSL-I, PBSL-II, and PBSL-III gained weight after preservation and after reperfusion, showing cell swelling. In PBSL-IV, weight decreased to almost normal after preservation was restored after reperfusion (Table 1 and Fig 1). Bile flow was not significantly different in the four groups examined (Table 2 and Fig 2). Liver enzymes were reduced significantly in PBSL-IV, showing better preservation (Tables 3, 4, and 5, Figs 3, 4, and 5).

Other parameters, including oxygen consumption and hydrostatic pressure, were not significantly different among the four groups.

DISCUSSION

Use of PEG in preservation solutions has been associated with a decreased incidence of rejection in clinical and experimental organ transplantation. The mode of action is uncertain but probably includes prevention of osmotic swelling and lipid peroxidation. It has also been suggested that PEG mitigates the immune response in both heart and liver in the whole animal. The addition of PEG to our preservation solution had a large impact on preservation of the liver. We used PEG at three different

Table 2. Bile Flow

<table>
<thead>
<tr>
<th></th>
<th>0–15 min</th>
<th>15–30 min</th>
<th>30–45 min</th>
<th>45–60 min</th>
<th>60–75 min</th>
<th>75–90 min</th>
<th>90–105 min</th>
<th>105–120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBSL-IV</td>
<td>5.34 ± 1.22</td>
<td>9.43 ± 1.4</td>
<td>8.45 ± 1.1</td>
<td>11.11 ± 0.98</td>
<td>11.94 ± 0.91</td>
<td>12.84 ± 0.89</td>
<td>9.71 ± 1.41</td>
<td>10.11 ± 1.28</td>
</tr>
<tr>
<td>PBSL-I</td>
<td>4.31 ± 1.2</td>
<td>9.44 ± 1.35</td>
<td>9.11 ± 1.2</td>
<td>10.94 ± 1.2</td>
<td>11.56 ± 1.33</td>
<td>12.66 ± 0.99</td>
<td>10.11 ± 1.1</td>
<td>9.83 ± 1.11</td>
</tr>
<tr>
<td>PBSL-II</td>
<td>4.95 ± 1.5</td>
<td>8.97 ± 1.61</td>
<td>9.09 ± 1.6</td>
<td>10.84 ± 1.5</td>
<td>10.97 ± 1.34</td>
<td>11.94 ± 1.43</td>
<td>9.45 ± 1.25</td>
<td>9.93 ± 1.21</td>
</tr>
<tr>
<td>PBSL-III</td>
<td>5.11 ± 0.98</td>
<td>9.42 ± 1.11</td>
<td>8.93 ± 1.4</td>
<td>11.03 ± 1.1</td>
<td>11.78 ± 1.22</td>
<td>13.03 ± 1.22</td>
<td>9.98 ± 1.24</td>
<td>10.05 ± 1.1</td>
</tr>
</tbody>
</table>

Data expressed as micrograms per 15 minutes per weight (grams) of liver. * P < .05 (one-way analysis of variance).
molecular weights to determine which is most appropriate for our solution. As judged by the lower weight gain and lower liver enzymes we chose to include the 20-mol/L PEG in our final solution.

In conclusion, polyethylene glycol (20 mol/L) provides much better colloid support in PBSL as judged by the decreased weight gain and lower liver enzyme release.

REFERENCES