Abstract

Objectives: Due to widespread exploitation of extended criteria donors, machine perfusion is emerging as an alternative to static cold storage for organ preservation. Hypothermic oxygenated machine perfusion has been associated with improved outcomes after liver transplant, both in laboratory and clinical settings. Here, we present our initial experience with hypothermic oxygenated machine perfusion, evaluating incidence of postreperfusion syndrome, early allograft dysfunction, and long-term biliary complications.

Materials and Methods: End-ischemic dual (hepatic artery and portal vein) hypothermic oxygenated machine perfusion was carried out for 150 to 200 minutes before organ implantation in 4 liver transplants considered at increased risk due to donor, recipient, or matching issues.

Results: No device malfunction occurred. Theatre logistics were minimally affected. Incidences of postreperfusion syndrome and early allograft dysfunction were 25% and 50%. At 6-month follow-up, all patients were alive with normal hepatic function and no evidence of ischemic cholangiopathy.

Conclusions: In our experience, hypothermic oxygenated machine perfusion appeared safe and logistically simple. Further studies are needed to assess the real value of this technique and to identify which subset of patients would benefit from its implementation.

Key words: Extended criteria donor, LiverAssist device, Organ preservation

Introduction

After some favorable experiences in the field of kidney transplantation, there has recently been a renewed interest in dynamic preservation techniques as applied to liver transplantation. This interest is justified by the need of reducing ischemia-reperfusion injury and assessing organ viability pretransplant in a scenario where so-called “extended criteria donors” are becoming everyday practice.

The question about what is the best dynamic preservation method is largely debated, with temperature being probably the most discussed point. Among several proposed techniques, hypothermic oxygenated machine perfusion (HOPE) has shown promising results, in both laboratory and clinical settings, where it has been associated with improved initial graft function and reduced incidence of ischemic cholangiopathy when using grafts from donors after cardiocirculatory determination of death.

Here, we report our initial experience with HOPE in 4 cases of liver transplant with grafts from brain-dead donors characterized by increased risk profile. Indications, technique, and results are discussed.

Materials and Methods

Machine perfusion use was considered in 4 cases characterized by a high-risk profile due to donor issues, severity of liver disease in the recipient, or both.

The LiverAssist device (OrganAssist, Groningen, The Netherlands) was used for all procedures, with cannulation of both portal vein and hepatic artery. Soon after organ arrival at the transplant center, a back-table was set up and the liver allograft was prepared in standard fashion while the device was primed. Timing of back-table preparation was coordinated with the start of recipient operation to allow at least 90 minutes of hypothermic perfusion.
At the end of back-table preparation, the superior mesenteric vein and celiac trunk were cannulated, leaving a side branch open to purge air from the circuit (splenic vein, when available, and gastroduodenal artery, respectively). The liver allograft was then weighed and connected to the perfusion device, taking care not to pump air or fatty debris into the graft. Hypothermic (10°C), oxygenated (O₂ pressure > 600 mm Hg) perfusion was started first through the portal vein and then through the hepatic artery. Pressure levels in the portal vein and hepatic artery circuits were set at 3 to 4 mm Hg and 25 mm Hg, with a continuous flow in the portal circuit and a pulsatile one in the artery. Modified low-viscosity University of Wisconsin solution was used for both perfusion circuits. During perfusion, lactate levels were monitored approximately every 30 minutes by taking perfusate samples for blood analysis. At the end of perfusion, the graft was flushed with about 1,000 mL of 5% chilled albumin while vascular anastomoses were performed. Liver biopsies were taken at the start and at the end of machine perfusion and after reperfusion in the recipient.

Study endpoints were the incidence of post-reperfusion syndrome and early allograft dysfunction (EAD), as well as 6-month patient and graft survival. Postreperfusion syndrome was defined as a drop in mean arterial pressure greater than 30% below the baseline, lasting at least 1 minute within 5 minutes after liver graft reperfusion. Severity of postreperfusion syndrome was classified according to Hilmi and associates. Early allograft dysfunction was defined as peak transaminases > 2000 IU/L, international normalized ratio > 1.7, or bilirubin > 10 mg/dL on postoperative day 7.

**Results**

Table 1 summarizes donor and recipient characteristics, as well as donor-recipient matching prognostic scores. Indications for HOPE included elevated donor age in the first 2 cases; high donor body mass index, hypernatremia, and elevated balance of risk score in the third; and high donor age × Model for End-Stage Liver Disease score in the fourth, where the recipient was a 25-year-old woman who had fulminant Wilson disease.

Mean duration of perfusion was 176 minutes (range, 150-200 min). There were no technical complications or device dysfunction incidents during perfusion. In all cases, we observed a progressive decrease of intrahepatic resistance in both the hepatic artery and portal vein circuits. Given the stable pressure monitored by the device, this resulted in a progressive increase of perfusate flow throughout perfusion (Figure 1). Macroscopically, we observed no swelling of liver allograft during or after perfusion. Oxygen concentration in the perfusate was adjusted to obtain a PaO₂ > 600 mm Hg (80 kPa). Serial perfusate gas analyses showed a variable trend in lactate concentration (Figure 2). However, we did not consider lactate level as a marker of liver graft function, and this parameter was not considered as a criterion to accept or discard the organ.

**Comparison between liver biopsies obtained before and after machine perfusion showed comparable histology with no sign of sinusoidal dilatation or other injury related to perfusion.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>Indication</th>
<th>Status</th>
<th>MELD</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>Cause of Death</th>
<th>ITU, d</th>
<th>D-MELD</th>
<th>BAR</th>
<th>DRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>25</td>
<td>PHCC + ALCI + HCC</td>
<td>Home</td>
<td>7</td>
<td>82</td>
<td>25</td>
<td>Cerebrovascular</td>
<td>5</td>
<td>571</td>
<td>3</td>
<td>2.54</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>32</td>
<td>PHCC + HCC</td>
<td>Home</td>
<td>8</td>
<td>79</td>
<td>34</td>
<td>Cerebrovascular</td>
<td>2</td>
<td>634</td>
<td>5</td>
<td>2.29</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>24</td>
<td>ALCI</td>
<td>Home</td>
<td>21</td>
<td>54</td>
<td>31</td>
<td>Cerebrovascular</td>
<td>3</td>
<td>1,127</td>
<td>18</td>
<td>1.30</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>25</td>
<td>Fulminant Wilson</td>
<td>ITU</td>
<td>35</td>
<td>61</td>
<td>25</td>
<td>Cerebrovascular</td>
<td>1</td>
<td>2,124</td>
<td>18</td>
<td>1.52</td>
</tr>
</tbody>
</table>

**Abbreviations:** ALCI, alcoholic cirrhosis; BAR, balance of risk score; BMI, body mass index; D-MELD, donor age × recipient model for end-stage liver disease score; DRI, donor risk index; HCC, hepatocellular carcinoma; ITU, intensive therapy unit; MELD, model for end-stage liver disease; PHCC, post-hepatitis C cirrhosis.
Interestingly, in 2 cases (cases 1 and 3), we observed a significant decrease in microvesicular steatosis during machine perfusion. Indeed, in these 2 cases, the degree of microvesicular steatosis was 40% in the biopsy before perfusion and 10% in the biopsy after perfusion (Figure 3). Liver graft histology at postperfusion biopsy showed no or mild macrovesicular steatosis in the first 3 cases, whereas there was 30% macrovesicular steatosis in case 4; macrovesicular steatosis was not modified by machine perfusion. Preservation injury was quantified as mild in the first 2 cases and moderate in the remaining.

Table 2 summarizes early and long-term outcomes. Reperfusion was smooth in the first 3 cases, with no change in mean arterial pressure and only a transitory increase in heart rate in case 2. In case 4, we observed a significant reperfusion syndrome, with a 40% drop of mean arterial pressure lasting about 5 minutes, which promptly recovered with volume expansion and inotropes. The overall impression of the surgical team was that, in all cases, reperfusion was quick and homogeneous, with liver grafts feeling softer than usual, also facilitating hemostatic maneuvers after reperfusion.

Concerning postoperative recovery, 2 patients (cases 1 and 3) had an uneventful course and were discharged on postoperative day 11 and 8, respectively. The second patient had a difficult transplant operation due to adhesions of previous abdominal surgery and was treated with perihepatic gauze packing and delayed abdominal closure, as previously described. He barely matched criteria for EAD for a bilirubin level of 10.7 mg/dL on day 7, but his liver function tests quickly normalized thereafter. He made a good recovery and was discharged on day 13. Patient 4 had a prolonged hospital stay, mostly due to her heavily compromised pretransplant status. She required protracted respiratory/hemodynamic support and dialysis due to renal failure before transplant in the intensive therapy unit. She received a steatotic graft and experienced a clinically significant EAD; however, her liver function slowly came back to normal and

### Table 2: Early and Long-Term Outcomes

<table>
<thead>
<tr>
<th>Patient</th>
<th>PRS</th>
<th>Peak AST, IU/mL</th>
<th>Peak ALT, IU/mL</th>
<th>Bili7, mg/dL</th>
<th>INR7</th>
<th>EAD</th>
<th>Days in ITU</th>
<th>Postop Stay</th>
<th>IC</th>
<th>Status*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>281</td>
<td>255</td>
<td>4.5</td>
<td>1.3</td>
<td>No</td>
<td>3</td>
<td>11</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>1089</td>
<td>336</td>
<td>10.7</td>
<td>1.3</td>
<td>Yes</td>
<td>5</td>
<td>13</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>72</td>
<td>348</td>
<td>1.8</td>
<td>1.18</td>
<td>No</td>
<td>2</td>
<td>8</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>Significant</td>
<td>2473</td>
<td>1260</td>
<td>13.6</td>
<td>1.15</td>
<td>Yes</td>
<td>22</td>
<td>46</td>
<td>No</td>
<td>Alive</td>
</tr>
</tbody>
</table>

*Refers to both patient and graft status at 6-month follow-up.

*Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bili7, bilirubin level on postoperative day 7; EAD, early allograft dysfunction; IC, ischemic cholangiopathy; INR7, international normalized ratio on postoperative day 7; ITU, intensive therapy unit; Postop, postoperative; PRS, preperfusion syndrome.
she was discharged in good condition and with no high-grade complication on postoperative day 46.

At 6-month follow-up, all patients and grafts were alive and well, with normal liver function and no clinical evidence of ischemic cholangiopathy.

Discussion

In past years, the widespread exploitation of suboptimal donors associated with inferior post-transplant outcomes has pushed the transplant community first to optimize donor-recipient matching and, more recently, to search for preservation method alternatives to static cold storage. In kidney transplantation, hypothermic nonoxygenated perfusion has been associated with reduced incidence of delayed graft function and better 1-year graft function in a paramount study by Moers and associates.\(^1\) In liver transplant, Guarrera and colleagues pioneered hypothermic nonoxygenated perfusion, observing a reduced incidence of EAD and biliary complications and a shorter hospital stay.\(^2\) The mechanism by which machine perfusion would exercise a protective effect is still speculative, but it seems to be related to better perfusion of sinusoids and microvascular peribiliary plexus, continuous delivery of metabolites, and better preservation of endothelial glycocalix through continuous paraphysiologic shear stress. More recently, HOPE was introduced, mainly by Groningen and Zurich groups.\(^6\)\(^,\)\(^13\) Compared with nonoxygenated hypothermic perfusion, the advantage of HOPE is to reduce mitochondrial oxidative stress with a down-regulation of mitochondrial respiration, which translates to a reduced production of reactive oxygen species at reperfusion. In experimental models, HOPE also seems to have an immunomodulatory effect and would allow a reduction of immunosuppression in the postoperative period.\(^14\) In the clinical setting, HOPE was used to recondition grafts from donors after cardiocirculatory determination of death and was associated with better initial function and reduced incidence of ischemic cholangiopathy.\(^15\)

We present here our initial experience with end-ischemic HOPE in 4 cases of liver transplant using extended criteria grafts from brain-dead donors. We found HOPE to be safe and logistically simple. No perfusion-related graft injuries were observed, as confirmed by serial biopsies and functional recovery after transplant. Even in the event of a device blockage, we felt that it would have been easy to convert hypothermic perfusion to static cold storage by simply disconnecting the liver from the device and placing it again in the bowl used for back-table. Theatre logistics was only minimally affected by the procedure, as the only modification compared with standard practice was the need to anticipate back-table start by approximately 90 minutes to allow enough time for the graft to be perfused.

One patient had significant postreperfusion syndrome, whereas the others had a smooth reperfusion, as reported by the anesthesia team. This could reflect washout of metabolites during perfusion, which could also be associated with the rather low transaminase peak observed after transplant. A constant impression of the surgical team, although hardly quantifiable, was that liver graft felt softer than usual after reperfusion. This could reflect decreased ischemia-reperfusion injury and probably goes along with the recruitment of a vascular bed observed during machine perfusion. Technically, this could be an important point, as graft stiffness frequently observed in the early phase after reperfusion could make hemostatic maneuvers more difficult of even impossible.

Overall, our outcomes were at least not inferior to what we would have expected with static cold storage. Due to the limitations of our study (limited case series lacking a control group), it is certainly not possible to make strong considerations concerning the real value of HOPE. However, our finding of a decrease of microvesicular steatosis is particularly intriguing and is in keeping with liver defatting observed during hypothermic perfusion in some animal studies.\(^16\) This confirms the capability of machine perfusion not only to reduce ischemia-reperfusion injury but also to “recondition” the liver graft during preservation, which should prompt further research especially about the use of steatotic grafts. Albeit logistically simple and safe, one major limit of HOPE in our experience appeared to be the impossibility of assessing liver graft function before transplant, thereby avoiding potentially unsuccessful transplants.

It is hoped that the 2 ongoing randomized controlled trials on hypothermic machine perfusion led by the University of Groningen (Dual Hypothermic Oxygenated Perfusion of DCD Liver Grafts in Preventing Biliary Complications After Transplantation; ClinicalTrials.gov NCT02584283)
and by the University of Zurich (Hypothermic Oxygenated Perfusion of Human Liver Grafts; ClinicalTrials.gov NCT01317342) will help define and quantify the benefits of HOPE.

In conclusion, these are exciting times in the field of organ preservation, which is likely to be the part of our practice that will change the most in the near future. Hypothermic oxygenated machine perfusion appears to be a safe, simple, and promising technique to reduce ischemia-reperfusion injury in liver transplant. Further studies will need to assess the real value of HOPE and to identify the subset of transplants that would benefit from this technique, as well as to evaluate it in relation to other dynamic preservation techniques like normothermic machine perfusion.

References