Studies on bile duct Injury and the protective role of oxygenated machine perfusion in liver transplantation
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CHAPTER 10

Dual Hypothermic Oxygenated Machine Perfusion in Liver Transplants Donated after Circulatory Death


* Both authors contributed equally

ABSTRACT

Experimental studies have suggested that end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) may restore hepatocellular energy status and reduce reperfusion injury in donation after circulatory death (DCD) liver grafts. The aim of this prospective case-control study was to assess the safety and feasibility of DHOPE in DCD liver transplantation. In consecutive DCD liver transplantations, liver grafts were treated with end-ischemic DHOPE. Outcome was compared with that in a control group of DCD liver transplantations without DHOPE, matched for donor age, donor warm ischemia time, and recipient Model for End-stage Liver Disease (MELD) score. All patients were followed for 1 year. Ten transplantations involving liver grafts treated with DHOPE were compared with 20 control procedures. There were no technical problems. All 6-month and 1-year graft and patient survival rates were 100 per cent in the DHOPE group. Six-month graft survival and 1-year graft and patient survival rates in the control group were 80, 67 and 85 per cent respectively. During DHOPE, median (IQR) hepatic adenosine 5'-triphosphate (ATP) content increased 11-fold, from 6 (3-10) to 66 (42-87) μmol per g protein (P = 0.005). All DHOPE-preserved livers showed excellent early function. At 1 week after transplantation peak serum alanine aminotransferase (ALT) and bilirubin levels were two-fold lower in the DHOPE group than in the control group (ALT: median 966 versus 1858 units/l respectively, P = 0.006; bilirubin: median 1.0 (0.7-1.4) versus 2.6 (0.9-5.1) mg/dl, P = 0.044). None of the ten DHOPE-preserved livers required retransplantation for non-anastomotic biliary stricture, compared with five of 20 in the control group (P = 0.140). This clinical study of end-ischemic DHOPE in DCD liver transplantation suggests that the technique restores hepatic ATP, reduces reperfusion injury, and is safe and feasible. RCTs with larger numbers of patients are warranted to assess the efficacy in reducing post-transplant biliary complications.

TRIAL REGISTRATION: The Netherlands national trial registry (www.trialregister.nl; trial ID NTR4493).
INTRODUCTION

Donation after circulatory death (DCD) liver grafts are increasingly used for transplantation in an attempt to overcome the discrepancy between the number of available donor livers and the number of patients waiting for a liver transplantation. In the US, transplantation of DCD livers accounted for 6% of all liver transplantations performed in 2013 (1), whereas in the UK and The Netherlands as much as 28% of liver transplantations in 2014 were performed with a DCD liver (2,3). The major drawback of DCD, compared to donation after brain death (DBD), is the inevitable period of warm ischemia in the donor after withdrawal of life support and circulatory arrest. This first period of warm ischemia and the subsequent cold ischemia during organ storage and transportation leads to the depletion of intracellular energy sources, such as adenosine-5-triphosphate (ATP), as well as other metabolic perturbations causing cellular injury and dysfunction (4,5). This damage is exacerbated by reperfusion injury of the liver graft and clinically manifested as an increased risk of complications and graft failure after transplantation (6). Compared to DBD liver transplantation, the most frequent complications after DCD liver transplantation are biliary complications (7,8). Posttransplant biliary complications include a spectrum of cholangiopathies causing cholestasis, jaundice and cholangitis, which may lead to graft loss. Non-anastomotic biliary strictures (NAS) have been reported in up to 30% of the patients after DCD liver transplantation, which is almost three times higher than in recipients of a DBD liver graft (6). Furthermore, NAS are the main cause of higher costs of DCD liver transplantation (9).

Experimental studies using animal models have suggested that machine preservation may provide better protection of liver grafts against ischemia-reperfusion injury than the traditional method of static cold storage (SCS) (10-16). Guerrera et al. were the first to report successful clinical transplantation of extended criteria DBD donor livers after ex situ hypothermic machine perfusion (4-6°C) via the portal vein and hepatic artery without active oxygenation (17-19). Dutkowski et al. subsequently reported the application of hypothermic (10°C) oxygenated perfusion in DCD liver transplantation (15-17,20,21). Although Dutkowski and coworkers applied active oxygenation of the perfusion fluid, they only perfused via the portal vein and not via the hepatic artery. It is well known, however, that blood supply to the bile ducts is largely dependent on the hepatic artery (22). Protection of the biliary tree is critical and single portal perfusion may not be sufficient to prevent ischemia-reperfusion injury of the bile ducts especially in DCD liver grafts. Dual hypothermic oxygenated machine perfusion (DHOPE) combines the advantages of the two above-mentioned techniques: active oxygenation and perfusion via both the portal vein and hepatic artery. We hypothesized that DHOPE is feasible and safe in resuscitating liver grafts procured from DCD donors. We, therefore, performed a first in man application of DHOPE in DCD liver transplantation.
Chapter 10

METHODS

Patient Selection
Between April 2014 and November 2014, ten consecutive patients (age ≥18 years) who received a DCD liver graft (Maastricht type 3 and donor body weight >40 kg) in our center were included in the study. Patients were excluded for the following reasons: mental conditions rendered them incapable of understanding the nature, scope and consequences of the trial; listed as high urgency; tested positive for HIV; pregnant or nursing; donor tested positive for hepatitis B or C; or the expected cold ischemia time exceeded eight hours. All livers were allocated according to the regular Eurotransplant rules based on ABO blood type compatibility and recipient model for end-stage liver disease (MELD) score (23). At the time of listing, patients gave informed consent to the possibility of receiving a DCD graft. At the time of donor liver offer, patients gave oral informed consent for machine preservation of the donor liver. The study protocol was approved by the institutional medical ethical committee (METc University Medical Center Groningen) and published in an open access national trial registry (www.trialregister.nl; trial-ID NTR4493).

Donor Organ Procurement and Preparation
Liver procurement was performed by one of the multi-organ recovery teams in the Netherlands. After circulatory death of the donor and a “no-touch” period of 5 minutes, the stand-by surgical team performed a midline thoracolaparotomy and aortic cannulation to perfuse the abdominal organs with at least 4 L of ice-cold (0-4 °C) University of Wisconsin cold storage (UW CS) solution (Belzer UW Cold Storage Solution, Bridge to Life Ltd, London, UK) to which 50,000 IU of heparin was added. A segment of supratruncal aorta was left attached to the celiac trunk for later cannulation (Figure 1A). The cystic duct was ligated and bile ducts were flushed with UW CS solution. Immediately after procurement, livers underwent an additional low pressure flush out through the portal vein with UW CS solution until the caval effluent was clear. Livers were transported using traditional SCS at 0-4°C in a box with melting ice. Upon arrival in our center, the back table procedure was performed and the portal vein and supratruncal aorta were cannulated. Before DHOPE, livers were flushed through the portal vein with 1 L ice-cold UW machine perfusion (UW MP) solution (Machine Perfusion Solution Belzer UW, Bridge to Life Ltd).

Dual Hypothermic Oxygenated Machine Perfusion
All livers underwent at least 2 hours of DHOPE using the Liver Assist device (Organ Assist, Groningen, the Netherlands). Machine perfusion was performed simultaneously with the recipient hepatectomy. In case of an unexpected difficult hepatectomy, DHOPE was prolonged (in three cases with 17, 19, and 52 minutes). The Liver Assist is a CE marked (European Union certification of safety, health and environmental requirements) device that allows pressure-controlled dual perfusion of the liver. Using two rotary pumps, a pulsatile arterial flow (60 bpm and amplitude of 20%) and a continuous portal flow is provided. Mean arterial pressure was set to 25 mmHg and portal vein pressure to 5 mmHg. Pressure settings were based on previ-
Dual Hypothermic Oxygenated Machine Perfusion in Liver Transplantation with Donation after Circulatory Death Grafts

ous studies and lower than physiological pressures to avoid shear stress-induced damage of the endothelium at low temperatures (10,13,16). Four liters of UW MP solution, supplemented with 3 mmol/L glutathione, were used as perfusion fluid at a temperature of 12°C. The perfusion fluid was oxygenated by two hollow fiber membrane oxygenators (100% oxygen at 500 mL/min), resulting in a pO$_2$ of $\geq$450 mmHg. No interventions were performed during machine perfusion.

Characteristics of DHOPE such as flow and resistance were assessed every ten minutes. Samples of perfusion fluid were collected every 30 minutes for immediate analyses of perfusate pH, partial pressure of gases, and concentrations of electrolytes and lactate using the ABL800 FLEX analyzer (Radiometer, Brønhøj, Denmark). Additional perfusate samples were centrifuged for 5 minutes at 2700 RPM at 4°C and stored at -80°C for later biochemical analyses. The concentration of thiobarbituric acid reactive substances (TBARS) was measured in the perfusion fluid as a marker of oxidative stress, as described previously (13). At the end of DHOPE, a perfusion fluid sample was collected for microbiological testing. Liver wedge biopsies were taken before and after DHOPE, snap frozen in liquid nitrogen and processed for measurement of ATP concentration as described previously (24).

Transplantation Procedure

Implantations were performed using the piggy-back technique without use of veno-venous bypass. Graft reperfusion was initiated after completion of the portal vein anastomosis, followed by construction of the arterial anastomosis, using the donor common or proper hepatic artery. Biliary reconstruction was performed using duct-to-duct anastomosis without a stent.

Control Group

Outcome data were compared with a matched control group. For each recipient of a DHOPE preserved graft two control patients were identified within a cohort of 61 patients who underwent a primary DCD liver transplantation between 2008 and 2014 in our center. The DCD livers of control patients were preserved with conventional SCS only. Matching criteria were based on known risk factors for graft survival: recipient age ($\pm$ 5 years), donor warm ischemia time ($\pm$ 5 minutes), and the MELD score (<22 or $\geq$23). Donor warm ischemia time was defined as the time interval between withdrawal of donor life support and initiation of aortic cold flush.

Posttransplant Outcome

The primary endpoint was graft survival at 6 months after transplantation. Graft survival was defined as the time interval between transplantation and retransplantation or death due to graft failure. Secondary endpoints were graft and patient survival rates at one year, technical safety of machine perfusion, microbiological testing of perfusion fluid, and postoperative complications. Serum markers of hepatobiliary injury and function (serum lactate, alanine aminotransferase (ALT), alkaline phosphatase (AlkP), gamma-glutamyl transferase (GGT), prothrombin time, and total bilirubin) were measured using standard biochemical assays. Other postoperative parameters assessed were length of ICU and hospital stay, and postoperative complications, including biliary complications such as NAS. NAS was defined as bile duct stenosis at
Figure 1. Characteristics of Dual Hypothermic Oxygenated Machine Perfusion and Posttransplant Markers of Hepatic Injury and Function

A. Liver graft with cannulas in the portal vein and suprarectal aorta during back-table, and before and after DHOPE. The asterisk indicates a wet sterile gauze protecting the arteries.

B. Flow rates were measured by flow sensors attached to the tubing of the perfusion device.

C. Perfusion pressure (mmHg) was measured by pressure sensors attached to the arterial and venous tubing. Vascular resistance was calculated using Ohms law and expressed as mmHg/mL/min/kg liver.

D&E. Levels of alanine aminotransferase (ALT), glucose and lactate were measured in perfusion fluid samples taken every 30 minutes during perfusion. SI conversion factors: to convert glucose to mmol/L, multiply by 0.0555; to convert lactate to mmol/L, multiply by 0.111.
After transplantation, serum lactate, prothrombin time, ALT, total bilirubin, gamma-glutamyl transferase (GGT), and alkaline phosphatase (AlkP) were assessed and compared between the two groups. Day 0 was determined as time interval between reperfusion and midnight.

G, SI conversion factor: to convert lactate to mmol/L, multiply by 0.111.
I, SI conversion factor: to convert bilirubin to mol/L, multiply by 17.104. AlkP indicates alkaline phosphatase; ALT, alanine aminotransferase; DHOPE, dual hypothermic oxygenated machine perfusion; GGT, gamma-glutamyl transferase; SCS, static cold storage. Shown are medians and error bars indicate interquartile ranges. Asterisks indicate a \( P < .05 \).
any location in the biliary tree (intra- or extrahepatic, but not at the site of the anastomosis) with cholestatic manifestations such as jaundice, cholangitis, or elevated laboratory tests, and in the presence of a patent hepatic artery. All recipients of a DHOPE preserved livers underwent magnetic resonance cholangiography 6 months after transplantation.

**Statistical Analyses**
Continuous variables are presented as medians and interquartile range (IQR). Categorical variables are presented as number and percentage. Continuous variables were compared between groups using the Mann-Whitney U test. Categorical variables were compared with the Pearson chi-square or Fischer exact test. Graft and recipient survival analyses were determined with the Kaplan-Meier method, and significance of survival differences was determined with the log rank test. A P value <0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS software version 22.0 for Windows (SPSS, Inc., Chicago, IL).

**RESULTS**
Donor and recipient characteristics of patients who received a DHOPE preserved DCD liver graft and their matched controls are summarized in Table 1. There were no significant differences in baseline characteristics between the two groups, except for a slightly lower body mass index in the DHOPE group.

**Graft and Patient Survival**
Six-month and one-year graft and patient survival rates were all 100% in the DHOPE group, whereas six-month graft survival and one-year graft and patient survival rates in the control group were 80%, 67% and 85%, respectively (P = 0.05 and P = 0.21, respectively) (Figure 2).

**Characteristics of DHOPE**
No technical problems or device malfunction occurred during machine perfusion. Microbiologic evaluation of the perfusion fluid revealed no evidence of microbial contamination. After a median cold ischemia time of 331 minutes (IQR 308-376 minutes), livers in the DHOPE group were perfused for a median duration of 126 minutes (IQR 123-135 minutes). This resulted in a total preservation time of 8.7 hours (IQR 7.8-9.9 hours) in the DHOPE group versus 8.4 hours (IQR 7.9-8.8 hours) in the control group (P = 0.45). Macroscopic appearance of a representative liver graft before and after DHOPE is shown in Figure 2A. Flows increased mostly during the first 30 minutes of DHOPE and reached a median portal venous flow of 365 mL/min and a median hepatic arterial flow of 84 mL/min after 2 hours (Figure 1B). In parallel, the vascular resistance decreased during the first 30 minutes and stabilized thereafter (Figure 1C). Median ALT concentration in perfusion fluid increased during the first 30 minutes of machine perfusion and decreased thereafter resulting in an ALT concentration of 207 U/L (IQR 134-878 U/L) at the end of DHOPE (Figure 1D). Lactate and glucose concentration in
Table 1: Donor and Recipient Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>DHOPE (n=10)</th>
<th>Control (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 (47-57)</td>
<td>53 (47-58)</td>
<td>0.91</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>5 (50%)</td>
<td>13 (65%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>23 (20-24)</td>
<td>25 (22-27)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>4 (40%)</td>
<td>6 (30%)</td>
<td></td>
</tr>
<tr>
<td>Post-anoxic brain injury</td>
<td>3 (30%)</td>
<td>5 (25%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>3 (30%)</td>
<td>9 (45%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Donor highest serum ALT (U/L)</td>
<td>88 (32-194)</td>
<td>35 (23-99)</td>
<td>0.28</td>
</tr>
<tr>
<td>Donor risk index&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.89 (1.47-2.19)</td>
<td>2.00 (1.73-2.20)</td>
<td>0.62</td>
</tr>
<tr>
<td>Time from circulatory arrest to cold perfusion (min)</td>
<td>15 (13-17)</td>
<td>16 (14-18)</td>
<td>0.62</td>
</tr>
<tr>
<td>Time from withdrawal of life support to cold perfusion (min)</td>
<td>27 (23-43)</td>
<td>32 (27-39)</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Preservation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of DHOPE (min)</td>
<td>126 (123-135)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total preservation time (min)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>521 (469-592)</td>
<td>503 (476-526)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Recipient Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>57 (54-62)</td>
<td>52 (42-60)</td>
<td>0.13</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>6 (60%)</td>
<td>11 (55%)</td>
<td>1.00</td>
</tr>
<tr>
<td>MELD score&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16 (15-22)</td>
<td>22 (17-27)</td>
<td>0.11</td>
</tr>
<tr>
<td>Indication for liver transplantation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcoholic cirrhosis</td>
<td>3 (30%)</td>
<td>2 (10%)</td>
<td></td>
</tr>
<tr>
<td>Nonalcoholic steatohepatitis</td>
<td>5 (50%)</td>
<td>2 (10%)</td>
<td></td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>1 (10%)</td>
<td>5 (25%)</td>
<td></td>
</tr>
<tr>
<td>Primary and secondary biliary cirrhosis</td>
<td>0</td>
<td>2 (10%)</td>
<td></td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>0</td>
<td>1 (5%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Hepatitis B or C cirrhosis</td>
<td>1 (10%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>0</td>
<td>4 (20%)</td>
<td></td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>0</td>
<td>3 (15%)</td>
<td></td>
</tr>
<tr>
<td>Familial amyloid neuropathy</td>
<td>0</td>
<td>1 (5%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; DHOPE, dual hypothermic oxygenated machine perfusion; MELD, model for end-stage liver disease.

<sup>a</sup> Data are expressed as No. (%) or median (interquartile range).

<sup>b</sup> Donor risk index is a validated tool to assess the risk of liver graft failure. 29

<sup>c</sup> Total preservation time was defined as the interval between start aortic cold flush in the donor and portal reperfusion in the recipient.

<sup>d</sup> MELD score was defined as the highest of laboratory derived MELD score or the (non) standard exception MELD score.
perfusion fluid also increased during the first 30 minutes and stabilized thereafter (Figure 2E). There was no significant increase in the concentration of TBARS in the perfusion fluid during DHOPE (data not shown).

Hepatic ATP concentration increased significantly during DHOPE from a median of 6 \( \mu \text{mol/g protein} \) to 66 \( \mu \text{mol/g protein} \) (IQR 42-87 \( \mu \text{mol/g protein} \); \( P = 0.005 \)). After graft reperfusion in the recipient, hepatic ATP levels were comparable to the levels at the end of DHOPE (Figure 3).

**Post Transplant Hepatobiliary Injury and Function**

Postoperative prothrombin time and serum lactate concentrations were comparable in both groups during the first 7 postoperative days (Figure 1F-G). Peak serum ALT levels were significantly lower in recipients of DHOPE preserved livers, compared to controls (median ALT 966 U/L vs. 1858 U/L; \( P = 0.01 \)) (Figure 1H). Also, serum bilirubin concentrations were significantly lower on postoperative day 7 in the DHOPE group, compared to controls (1.0 mg/dL [IQR 0.7-1.4 mg/dL vs. 2.6 mg/dL [IQR 0.9-5.1 mg/dL]]; \( P = 0.04 \)) (Figure 1I). Serum values of ALT, GGT, AlkP, and bilirubin at 30 days after transplantation were lower in the recipients of DHOPE preserved livers compared to the control group (median ALT 17 vs. 51 U/L \( P = 0.02 \), GGT 74 vs. 176 U/L \( P = 0.05 \), AlkP 115 vs. 182 U/L \( P = 0.02 \), bilirubin 0.5 vs. 1.0 mg/dL \( P = 0.02 \)) (Figure 1H-I).

![Graft and Patient Survival Rates](image)

**Figure 2. Kaplan-Meier Curves of Graft and Patient Survival Rates within the First Year After Transplantation**

Indications for retransplantation in the control group were: NAS in three patients; necrotic bile ducts in two patients; and hepatic artery thrombosis in one patient. Causes of death in the control group were: recurrence of angiosarcoma in one patient; pneumonia as complication of treatment for hemophagocytic syndrome in one patient; and hemorrhagic shock due to intrathoracic bleeding after thoracentesis for pleural effusion in one patient. DHOPE indicates dual hypothermic oxygenated machine perfusion.
Post Transplantation Outcome
There were no significant differences in kidney function, length of ICU or hospital stay, and the incidence of postoperative complications, except for postreperfusion hypokalemia, which developed in three recipients of a DHOPE liver (Table 2). One recipient of a DHOPE preserved liver developed NAS in segment 2 and 3 of the liver. In contrast, 7/20 (35%) of the matched control patients developed NAS. The recipient of a DHOPE liver who developed segmental NAS was successfully treated by endoscopic stenting and removal of a biliary cast.

DISCUSSION
This first clinical series of end-ischemic DHOPE in DCD liver transplantation suggests that this method of liver machine preservation is safe and feasible. DHOPE seems to provide better preservation of DCD liver grafts, resulting in a reduction of ischemia-reperfusion injury and better early graft function, compared to SCS alone. Graft and patient survival rates after transplantation of DHOPE preserved DCD livers were 100% during a 12 month follow up period. One-year graft and patient survival rates in the matched controls were 67% and 85%, respectively.

There were no technical failures or device related events, no evidence of bacterial contamination of perfusion fluid, and no signs of oxidative stress during the perfusion. After transplantation, peak serum ALT levels and serum bilirubin levels at one week were significantly lower in recipients of a DHOPE preserved DCD liver, compared to controls. A high peak ALT after DCD liver transplantation has previously been identified as an independent risk factor for the development of NAS (25,26). Only one of the ten patients who received a DHOPE preserved liver in our study developed NAS, limited to the left lateral segments of the liver.

Figure 3. Hepatocellular Energy Levels
ATP indicates adenosine-5-triphosphate; DHOPE, dual hypothermic oxygenated machine perfusion; SCS, static cold storage. Shown are medians and error bars indicate interquartile ranges. Asterisks indicate a P <.05.
Table 2: Posttransplant Outcome

<table>
<thead>
<tr>
<th>Variable</th>
<th>DHOPE (n=10)</th>
<th>Control (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak serum creatinine ≤1 week (mg/dL)</td>
<td>1.2 (1.0-1.5)</td>
<td>1.0 (0.8-1.2)</td>
<td>0.37</td>
</tr>
<tr>
<td>Length of ICU stay (d)</td>
<td>2 (2-6)</td>
<td>2 (1-5)</td>
<td>0.47</td>
</tr>
<tr>
<td>Length of hospital stay (d)</td>
<td>22 (16-33)</td>
<td>23 (15-32)</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypokalemia (&lt;3.5 mEq/L) after reperfusion</td>
<td>3 (30%)</td>
<td>0 (0%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Primary non-function&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>-</td>
</tr>
<tr>
<td>Relaparotomy&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3 (30%)</td>
<td>7 (35%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Renal failure requiring hemodialysis</td>
<td>1 (10%)</td>
<td>2 (10%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hepatic artery thrombosis</td>
<td>0 (0%)</td>
<td>2 (10%)</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Biliary complications</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anastomotic biliary stricture</td>
<td>2 (20%)</td>
<td>3 (16%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.00</td>
</tr>
<tr>
<td>Biliary cast formation</td>
<td>3 (30%)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3 (15%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.37</td>
</tr>
<tr>
<td>Non-anastomotic biliary strictures</td>
<td>1 (10%)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>7 (35%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.21</td>
</tr>
<tr>
<td>Massive biliary necrosis</td>
<td>0 (0%)</td>
<td>2 (10%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Retransplantation for biliary complications</td>
<td>0 (0%)</td>
<td>5 (25%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Abbreviations: DHOPE, dual hypothermic oxygenated machine perfusion; ICU, intensive care unit.

<sup>a</sup> Data are expressed as No. (%) or median (interquartile range)

<sup>b</sup> SI conversion factor: To convert creatinine to mol/L, multiply by 88.4.

<sup>c</sup> SI conversion factor: to convert potassium to mmol/L, multiply by 1.

<sup>d</sup> Primary non-function was determined as retransplantation or death within 7 days after transplantation.

<sup>e</sup> Indications for relaparotomy in DHOPE group: intra-abdominal blood loss due to diffuse oozing in one patient; removal of surgical gauzes used for packing to control diffuse oozing during transplantation in one patient; and biliary anastomotic leakage in one patient. Indications for relaparotomy in control group: intra-abdominal blood loss due to diffuse oozing in one patient; removal of surgical gauzes used for packing to control diffuse oozing during transplantation in four patients; biliary anastomotic leakage in two patients.

<sup>f</sup> One patient had a combination of an anastomotic biliary stricture and biliary cast formation. Two patients had biliary cast formation as well as non-anastomotic biliary strictures. One patient had non-anastomotic biliary strictures and later also developed an anastomotic biliary stricture.

<sup>g</sup> One patient had a combination of biliary cast formation and non-anastomotic biliary strictures.

This was successfully treated with endoscopic stenting and did not require retransplantation. In contrast, NAS were noted in 7 of the 20 (35%) matched controls. A potentially beneficial effect of hypothermic oxygenated machine perfusion on the occurrence of biliary complications after DCD liver transplantation has previously been reported by Dutkowski <i>et al.</i> (8,21). These investigators observed no intrahepatic biliary strictures in a series of 25 DCD liver transplants after end-ischemic HOPE. Formal evidence for the efficacy of hypothermic oxygenated machine perfusion in reducing the incidence of biliary complications after DCD liver transplantation should come from randomized clinical trials. Based on the current favorable results, we have recently initiated a multicenter randomized clinical trial comparing end-ischemic DHOPE with SCS alone in DCD liver transplantation. The primary endpoint of this study will be the incidence of NAS within 6 months after transplantation (ClinicalTrials.gov Identifier: NCT02584283).
One of the main benefits of a short period of DHOPE after SCS of DCD livers appears to be the restoration of intrahepatic energy sources. As a result of the periods of warm and cold ischemia DCD livers become severely ATP depleted. In the current study, intrahepatic ATP levels increased 10-fold during DHOPE. These results are in accordance with experimental studies that have shown that oxygenated machine perfusion enables mitochondria to generate ATP even at low temperatures (13,16,27). Restoration of ATP levels reduce the cellular oxygen debt, resulting in reduced production of radical oxygen species and damage associated molecular pattern molecules after warm reperfusion in the recipient (10,11,20). The downstream effects of this are reduced activation of Kupffer cells and endothelium, limiting graft ischemia-reperfusion injury and resulting in a down regulation of the immune response after transplantation (14-16).

A potential risk of combined portal and arterial perfusion of a donor liver is mechanical damage that may occur to the hepatic artery and could cause a higher incidence of hepatic artery thrombosis after transplantation. For this reason, Dutkowski et al. only used portal vein perfusion (8,13,21). On the other hand, biliary complications are the main obstacle for wider utilization of DCD livers and based on the dominant arterial vasculature of the biliary tree, single portal perfusion may not provide optimal preservation of the bile ducts and their vasculature (22). We have avoided manipulation and cannulation of the hepatic artery by leaving a segment of suprarenal aorta attached the donor liver. After machine perfusion, this part of the arterial vasculature was cut off and the donor common or proper hepatic artery was used for anastomosis. None of the patients in the present study developed hepatic artery thrombosis. Guarrera et al. have also used combined portal and arterial perfusion without evidence of an increased risk of arterial complications (18,19).

We have recently reported on the feasibility and potential benefits of end-ischemic normothermic machine perfusion of human donor livers (24,28). In contrast to hypothermic machine perfusion, normothermic liver perfusion enables an ex situ functional assessment. This may facilitate the identification of transplantable donor livers that would otherwise have been declined because of a high risk of primary non-function. Hypothermic oxygenated machine perfusion does not allow functional assessment of the liver prior to transplantation, but rather aims at reducing graft dysfunction and complications after transplantation. In this respect, the various types of machine perfusion at different temperatures may prove to be complementary. A major advantage of hypothermic machine perfusion may be its relative simplicity and safety.

CONCLUSION

This study suggests that end-ischemic combined portal and arterial hypothermic oxygenated machine perfusion is feasible and safe in resuscitating DCD liver grafts prior to transplantation. DHOPE resulted in a restoration of hepatic ATP content and was associated with a reduction in ischemia-reperfusion injury as well as better hepatobiliary function after transplantation. These favorable results provide a rationale to start randomized clinical trials with larger numbers of patients to study the efficacy of DHOPE in reducing (biliary) complications after DCD liver transplantation.
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PART C

Addendum