Effects of Subnormothermic Perfusion Before Transplantation for Liver Grafts from Donation After Cardiac Death: A Simplified Dripping Perfusion Method in Pigs

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ABSTRACT

Background. Liver transplantation from donors after cardiac death (DCD) provides a solution to the donor shortage. However, DCD liver grafts are associated with a high incidence of primary graft nonfunction. We investigated the effectiveness of subnormothermic porcine liver perfusion, before transplantation from DCD, on graft viability.

Methods. Landrace pigs (25–30 kg) were randomly allocated to 3 groups (5 per group): heart-beating (HB) graft, transplanted after a 4-hour period of cold storage (CS); DCD graft, retrieved 20 minutes after apnea-induced cardiac arrest (respiratory withdrawal) and transplanted after a 4-hour period of CS; and subnormothermic ex vivo liver perfusion (SELP) graft, retrieved in the same manner as the DCD graft but perfused with a subnormothermic oxygenated Krebs-Henseleit buffer (21–25°C, 10–15 cm H_2O) for 30 minutes in a simplified dripping manner, without a machine perfusion system, after the 4-hour period of CS, and subsequently transplanted.

Results. Although all animals in the HB group survived for >7 days, all animals in the DCD group died within 12 hours after transplantation. In the SELP group, 2 recipients survived for >7 days and another 2 recipients were killed on day 5. The survival rate was significantly better for SELP than for DCD grafts ($P = .0016$). The values of tumor necrosis factor α were not significantly different between the SELP and HB groups. Preserved structure of the parenchyma was observed in the SELP group on histologic examination.

Conclusions. A simplified subnormothermic perfusion before liver transplantation is expected to improve graft viability and survival.

LIVER transplantation (LT) is a well established treatment for end-stage liver diseases. However, the shortage of donors for transplantation is a serious problem, with the use of livers from donors after cardiac death (DCD) now being necessary. In fact, DCD grafts currently contribute to as much as 20% of the liver donor pool in some European countries [1]. However, the use of DCD livers is associated with a high incidence of primary graft nonfunction (PNF) [2,3] and biliary complications [4]. These limitations of using DCDs need to be resolved for successful LT programs.

Machine perfusion (MP) can improve the viability of DCD grafts by supplying oxygen and nutrients. MP also provides a way to assess the viability of a graft before LT. In fact, normothermic MP has recently begun to be used for DCD LT in humans [5]. However, in contrast to normothermic and hypothermic conditions, subnormothermic

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Table 1. Recipient Survival After Liver Transplantation

Group	Survival	Cause of Death
HB ($n = 5$)	>7 days	Killed
	>7 days	Killed
	>7 days	Killed
	>7 days	Killed
	>7 days	Killed
DCD ($n = 5$)	<12 hours	Primary graft nonfunction
	<12 hours	Primary graft nonfunction
	<12 hours	Primary graft nonfunction
	<12 hours	Primary graft nonfunction
	<12 hours	Primary graft nonfunction
SELP ($n = 5$)	>7 days	Killed
	>7 days	Killed
	5 days	Ileum perforation, peritonitis
	5 days	Pneumonia
	<12 hours	Primary graft nonfunction

Abbreviations: HB, heart-beating donation group; SELP, subnormothermic ex vivo liver perfusion; DCD, donation after cardiac death.

perfusion does not require a complex MP system, such as a heating device, which makes transportation of the graft between different clinical institutions difficult.

The Zurich group demonstrated that 1-hour short-term hypothermic oxygenated perfusion for DCD liver grafts was effective [6–8]. We previously reported the benefit of short oxygenated warm perfusion, provided for 30 minutes before cold storage (CS), for improving the viability of the DCD graft [9–13]. In the present study, we sought to extend our research toward the design of a more simplified perfusion method to facilitate clinical application. We investigated the effectiveness of subnormothermic oxygenated perfusion for DCD porcine livers in a dripping manner for 30 minutes, without using of MP and oxygen carriers, before LT.

METHODS

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health, and the study was approved by the Animal Care and Use Committee of Tohoku University.

Experimental Design

Fifteen Landrace pigs, weighing 25–30 kg, were randomly allocated into 3 experimental groups to receive 1 of 3 types of liver grafts, with 5 animals in each group: heart-beating (HB) graft, transplanted after a 4-hour period of cold storage (CS) in University of Wisconsin (UW) solution (Dupont Pharmaceuticals, Wilmington, Delaware); DCD graft, retrieved 20 minutes after induced cardiac arrest and transplanted after a 4-hour period of CS in UW solution; and subnormothermic ex vivo liver perfusion (SELP), where the graft was retrieved in the same manner as the DCD graft but perfused with a subnormothermic oxygenated Krebs-Henseleit buffer (21–25°C, 10–15 cm H_2O) for 30 minutes in a dripping manner after the 4-hour period of CS in UW solution and subsequently transplanted.

Surgical Procedure for Donors

The pigs were anesthetized with the use of intramuscular medetomidine (Nippon Zenyaku Kogyo Co, Fukushima, Japan; 0.1 mg/kg) and midazolam (Teva Pharma Japan, Tokyo, Japan; 0.5 mg/kg). Anesthesia was maintained with the use of an isoflurane (Abbvie GK, Tokyo, Japan)–oxygen mixture and intravenous buprenorphine hydrochloride (Otsuka Pharmaceutical Co, Tokyo, Japan). We have previously provided a detailed report of our surgical procedures [14]. Briefly, the common bile duct was cannulated, and after administering of heparin (AY Pharmaceuticals Co, Tokyo, Japan; 300 U/kg) intravenously, the splenic vein and abdominal aorta were cannulated. In the DCD and SELP groups, the respirator was stopped, followed by administration of Vecuronium bromide (MSD KK, Tokyo, Japan) and phrenotomy to induce cardiac arrest, which was defined as systolic blood pressure <40 mm Hg. At 20 minutes after arrest, liver grafts were perfused with a cold lactate Ringer solution, followed by perfusion with 1,000 mL UW solution administered through the portal vein and abdominal aorta. Grafts were retrieved immediately after flushing and preserved in UW solution for 4 hours. In the HB group, we performed cross-clamping of the abdominal aorta and retrieved liver grafts with the use of the same procedure but without inducing cardiac arrest.

Dripping Perfusion Method

In the SELP group, following the 4-hour period of CS, grafts were perfused with a subnormothermic oxygenated Krebs-Henseleit buffer (21–25°C) via the portal vein and without the use of MP and oxygen carriers. The subnormothermic perfusate was prepared at 21–25°C and dripped to perfuse on the graft for 30 minutes, while controlling portal pressure to 10–15 cm H_2O, which was measured by means of cannulation into the branch of the portal vein. Oxygen gas was bubbled into the buffer, creating a pO_2 of 500–550 mm Hg. The graft was immediately transplanted after the perfusion.

Surgical Procedures for Recipients

Recipient pigs were anesthetized in the same way as the donors. We performed laparotomy, and the liver was removed. After administering heparin (300 U/kg) intravenously, a venous-venous bypass (passive shunt) was created during the anhepatic phase, with the use of Anthron bypass tubes (Toray, Tokyo, Japan), from the splenic vein to the left jugular vein and from the infrahepatic vena cava to the right jugular vein [15]. Liver grafts were implanted orthotopically, and the grafts were reperfused with portal blood after completion of the anastomosis between the suprahepatic vena cava and the portal vein. After infrahepatic vena cava and hepatic artery reconstruction [14], the bile duct was drained through an external tube fistula.

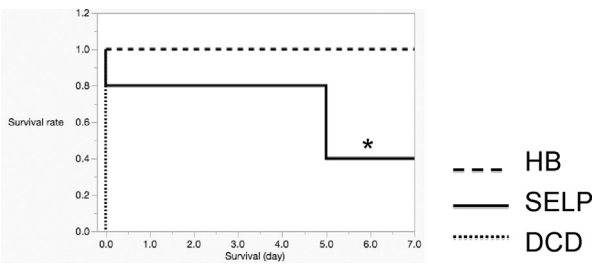

Fig 1. Survival curve of recipients after liver transplantation. *The 7-day survival rate was significantly higher in the SELP than in the DCD group ($P = .0016$, Kaplan-Meier log-rank test). Abbreviations: HB, heart-beating donation group; SELP, subnormothermic ex vivo liver perfusion; DCD, donation after cardiac death.