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Applicability of Combined Use of Extracorporeal Support and Temperature-Controlled Machine Perfusion Preservation for Liver Procurement of Donors After Cardiac Death in Pigs.

M.Hagiwara, N.Matsuno, L.T.Meng, M.Furukori, K.Watanabe, T.Shonaka, K.Imai, H.Obara, Y.Nishikawa, H.Furukawa

Applicability of combined use of extracorporeal support and temperature controlled machine perfusion preservation for DCD liver procurement

Masahiro Hagiwara[a], Naoto Matsuno[a], Ling tong Meng[a], Mariko Furukori[a],

Kenji Watanabe[a], Tatsuya Shonaka[a], Koji Imai[a], Hiromichi Obara[b],

Yuji Nishikawa[a], Hiroyuki Furukawa[a]

[a] ,Asahikawa Medical University, Department of Surgery

[b] Tokyo Metropolitan University, ,Mechanical Engineering

Address correspondence to: Naoto Matsuno, M.D., PhD
Department of Surgery, Asahikawa Medical University
2-1 Midorigaoka, Asahikawa, Hokkaido 078- 8501, Japan,
TEL: +81-166-68-2503 FAX: +81-166-68-2193
E-mail: mtn@asahikawa-med.ac.jp

Figure 3, Table 0

M.H: fudoushin_hms@yahoo.co.jp Data acquisition, Participate in the experiment, Data analysis and interpretation and Drafting the article

N.M: mtn@asahikawa-med.ac.jp Participate in the experiment and revising it critically for important intellectual content

M.F: marichiictan@gmail.com; DATA acquisition, Participate in the experiment, Data analysis and interpretation and Drafting the article

L.M: 496042328@qq.com; Data analysis and interpretation, Participate in the experiment

K.W: knj-wata@asahikawa-med.ac.jp; Participate in the experiment

T.S: shonaka@asahikawa-med.ac..jp Participate in the experiment

K.I: kimai@asahikawa-med.ac.jp; Participate in the experiment

H.O: obara@tmu.ac.jp Participate in the experiment as a engineer

Y.N: nishikwa@asahikawa-med.ac.jp; Histopathological analysis, Participate in the experiment

H.F: f-hiro@asahikawa-med.ac.jp; Revising it critically for important intellectual content

Key words: Donation after circulatory death, machine perfusion preservation, subnormothermic preservation,

Abbreviation machine perfusion: MP, warm ischemic time: WIT,

aspartate aminotransferase: AST, lactate dehydrogenase: LDH,

donation after cardiac death: DCD

Highlights:

Highlights of this study is focus on the organ preservation temperature with use of machine.

The objective of this study is to determine the benefits of the extracorporeal membrane oxygenation (ECMO) and subnormothermic machine preservation with rewarming in a large animal model of donation after crdiac death (DCD) liver grafts. As a results, the combined use of in situ subnormothermic ECMO and machine preservation with rewarming is more essential for the recovery and resuscitating function of DCD liver grafts.

Abstract

Utilization of grafts from donors after cardiac death (DCD) would greatly contribute to the expansion of the donor organ pool. The objective of this study is to determine the benefits of the extracorporeal membrane oxygenation (ECMO) and subnormothermic machine perfusion (MP) with rewarming in a large animal model of DCD liver. 【Methods】After cardiac arrest, the abdominal aorta and the inferior vena cava cannulated and connected to a ECMO circuit. Porcine livers were perfused in situ with ECMO at 22 ° C for 60min after 60 min of cardiac death then, the livers were perfused for 4 hours by MP as a graft viability test. Group 1: Non in situ ECMO and grafts was preserved hypothermic MP. Group 2: Non in situ ECMO and grafts was preserved subnormothermic rewarming MP. Group3: ECMO and subnormothermic rewarming MP. To assess potential methods

and effect, effluent enzyme was measured. Portal vein and hepatic artery pressure during MP were evaluated. 【Results】Effluent enzyme of AST.ALT and LDH as a viability marker were significantly in low (AST: 2899,2292 and 972IU/L. ALT; 134,140 and 72 IU/L. LDH: 4354, 4455 and 1855IU/L in each group respectively.) Portal vein and hepatic artery pressure during preservation came down smoothly in Group 3 compared to Group 1. 【Conclusion】 The combined use of in situ subnormothermic ECMO and machine preservation with rewarming is more essential for the recovery and resuscitating function of DCD liver grafts.

Introduction

Use of grafts from donors after cardiac death (DCD) would greatly contribute to the expansion of the donor organ pool. However, this requires the development of novel preservation methods to recover the organ from changes due to warm ischemic time (WIT). In 2007, the group of Barcelona described the first 10 human liver transplant recipients who received a DCD liver procured according to normothermic extracorporeal membrane oxygenation (NECMO) protocol. The main factor that limits further expansion of the use of these unexpected DCD livers for transplant is the fact that cold storage (CS) is an inadequate methods of maintaining their viability ex vivo (1) .Experimental studies have demonstrated that even brief periods of cold preservation will cause injury to hepatocytes, Kupffer cells and endothelial cells in DCD liver(2). Considering the

limitations of the hypothermic preservation of DCD liver grafts, research on the transition with rewarming up to 22℃ preservation with use of temperature controlled machine has been reported (3). Rewarming using a well-controlled system was demonstrated the protective effects for the function of hepatocytes and sinusoidal injury during machine perfusion (MP) preservation. The objective of this study is to determine whether subnormothermic machine perfusion with rewarming (RMP) improves upon the beneficial effects of the preservation of livers subjected to an extended period of warm ischemia followed by subnormothermic ECMO.

Methods

Six outbred male weanling pigs (20-25 Kg) were used. Cardiac arrest was induced by KCL injection. After cardiac arrest, the abdominal aorta and the inferior vena cava were cannulated and with a 16 Fr cannula (Flexmaite TWN-16, Toyoboseki, Japan). These were connected to a cardiopulumonary bypass circuit (ECMO) after 500ml of sodium biocarbonate,500 mL low molecular dextran and 500 mL of Voluven (Fresenius Kabi. Bas,Humburg, Germany).ECMO was consisted of extracorporeal circuit (HAP-1, Nikkiso, Japan) and membrane oxygenation system(hp0-06, RHF-C,MERA, Japan) . Furthermore, dialyzer (PNP inafinal, Nikkiso,Japan) was added to this system directly (Figure 1,2), Sixty minutes after cardiac arrest, ECMO was started and run for 60 minutes, with the pump for maintained at 1.2L/ml and the temperature 22 ° C .At the end of ECMO, the liver was perfused

with 1L of cold Euro Collins solution through artery, placed in CS 4℃. After the liver was removed the portal vein and celiac trunk were cannulated, and the liver was connected to the machine. Then, the liver was perfused with machine with rewarming up to 22~25℃ for 4 hours. Machine preservation was performed as a graft viability assessment(Figure 3).

Ex vivo liver preservation machine

The system has been previously reported by our group (4). Briefly, system consists of three circulating systems for a portal vein, a hepatic artery and the maintenance of a perfusion solution. Each system for the portal vein or the hepatic artery has a roller pump ,a flow meter and a pressure sensor . Both systems were able to control each, and a flow rate and a pressure which were continuously monitored. The maintenance system has a filter unit to remove cellular wastes and a heat exchanger unit to maintain a temperature of the solution. And the temperature of the organ chamber was controlled with the supplied water of this heat exchanger.The flow rate of the portal vein was 0.5 ml/min/g liver and the calculated pressure at the entrance of the portal vein was 10 mmHg and the flow rate of the hepatic artery was 0.2 ml/min/g liver and the calculated pressure at the entrance of the hepatic artery was 30 mmHg.

Experimental groups

Group 1: Non in situ ECMO and grafts was preserved hypothermic MP.

Group 2: Non in situ ECMO and grafts was preserved subnormothermic rewarming MP.

Group3: ECMO and subnormothermic rewarming MP.

Graft viability assessment

Previously, effluent was obtained after 2 hours of MP preservation to analyze the aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels and hyaluronic acid (HA) in perfusate during preservation were reported as beneficial biomarker for ischemic damage by our group(5). In this study, to assess effluent enzymes, AST, LDH and HA during preservation was measured for four hours. Portal vein and hepatic artery pressure during MP were evaluated.

Liver biopsy specimens were obtained 2 hour after preservation.

Statistical Analysis

The SPSS software program was used for the statistical analysis

Ethical Considerations

All experimental procedures were approved by our institutional animal ethics committee (permission No. 14172).

Results

The PH during subnormothermic ECMO were significantly recovered to normal level in Group 3. Effluent enzyme of AST,ALT and LDH as a viability marker were significantly in low (AST: 2899, 2292 and 972IU/L. ALT: 134,140 and 72 IU/L. LDH: 4354, 4455 and 1855IU/Lin each group respectively.) Portal vein and hepatic artery pressure during preservation came down smoothly in Group 3 compared to Group 1(Figure 4). Liver biopsy showed remarkable enlarged Disse spaces space was demonstrated in Group 2. The mild fatty degenerate and swelling of hepaotcytes were shown in Group 3 (Figure 5).

Discussion

In March 1995, an international workshop for NHBD was held in Maastricht, Netherlands. DCDs had been classified as the Maastricht classification (6). Categories 1, 2, and 4 include uncontrolled DCDs, and category 3 includes controlled DCDs. DCDs have come to represent the fastest growing proportion of the donor pool. After successful use of DCD kidney grafts for clinical transplantation, interest has moved toward using extrarenal organs such as the liver, pancreas, and lungs (7). However, in the early phase, liver transplantations from DCDs did not always show favorable

post-transplantation results. The development of ischemic biliary stricture is a major source of morbidity after DCD liver transplantation. Retransplantation is also associated with a significantly higher mortality risk. On the other hand, the concept for DCD graft has been changed and reported in recent years. DCDs have already suffered severe tissue damage secondary to hypoxia and hypoperfusion before the initial period of warm ischemia. Additional cold storage damage to the organ caused by hypothermic conditions may limit the ability to improve cellular function because metabolic activity is decreased in the cold storage The use of normothermic extracorporeal membrane oxygenated (NECMO) perfusion is based on experimental studies which have shown that the recirculation of oxygenated blood at 37℃ improves the cellular energy load, reduces tissue injury, and improves the posttransplantation graft function in livers damaged by the period of warm ischemia caused by cardiac arrest (8.9). In 2002, the Hospital Clinic in Barcelona developed a clinical protocol to resuscitate organs from donors and to maintain viability for transplantation (1). NECMO is used to reperfuse and oxygenate abdominal organs after cardiac arrest while the potential DCD is evaluated and consent for organ donation is obtained. In 2007, the first 10 human liver transplantations were performed with uncontrolled DCDs in which the donor was maintained with NECMO before organ retrieval. Ten DCD livers were transplanted with

only 1 graft lost to PNF and 1 to hepatic artery thrombosis. On the other hands, the use of normothermic machine perfusion (NMP) by avoiding cold ischemia, not only improves the viability of damaged grafts but may also offer the opportunity to functional recover (10). Fondevila et al reported the remarkable effects of combined use of in situ NECMO and NMP in pig 90 minutes of DCD liver transplantation model (11). The advantage of normothermic preservation,including the use of NECMO, is the ability to overcome the disadvantaged aspects of hypothermic cellular physiology. NECMO initiates the processes of energy repletion and cellular repair, whereas subsequent NMP provides the physiological conditions and substrates necessaly for continued graft improvement during ex vivo preservation(10,11). However, the use of blood-based perfusates may increase the risk of microvascular failure and sinusoidal plugging and bacterial growth. Normothermic preservation requires full metabolic support with a large machine.. Therefore, achieving normothermic liver preservation remains troublesome and expensive. The reality of clinical organ retrieval might require a period of cold preservation due to transport between institutions. Some studies have investigated the perfusion temperature. For example, subnormothermic MP performed at 20C resulted in reduced vasoconstriction, as well as lower metabolic requirements in DCD (12) and steatotic (13) rat models. A temperature of 22~25℃ is considered to

allow a minimum level of metabolic activity and has preventative effects for cell lysis. Taking advantage of MP, the rewarming preservation method for DCD liver grafts was applied by our group (13, 14).

Matsuno et al successfully transplanted porcine livers with 60 minutes of WIT plus 4 hours of total ischemic time by rewarming preservation from 4℃ to 22℃ using MP (14). This study demonstrated that the use of in situ subnormothermic ECMO after an extended period of warm ischemia significantly improves outcome. In addition, it shows that subsequent subnormothermic ex vivo machine perfusion with rewarming significantly enhances the benefit of in situ ECMO. By providing continuous minimum level of physiological metabolism and avoiding long cold ischemia RMP may recover the function of damaged grafts. Further study are needed to determine what the extent of the clinical application would be allowed for this combined technology.

In conclusion, the combined use of in situ subnormothermic ECMO and machine preservation with rewarming is more potential for the recovery and resuscitating function of DCD liver grafts.

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Figure Legends

Figure 1: Experimental design

Figure 2: Subnormothermic extracorporeal membrane oxygenation with direct dialyzer -1

Figure 3; Subnormothermic extracorporeal membrane oxygenation with direct dialyzer -2

Figure 4; Evaluation by effluent enzymes after machine perfusion
LDH,AST level were decreased in subnormothermic ECMO with subnormothermic liver perfusion preservation

Figure 5: Liver biopsies after subnormothermic liver perfusion.
A ; normal liver tissue, B: Group 1, C; Group 2, D; Group 3
Group 3 was not seen enlargement of Disse space and showed better integrity

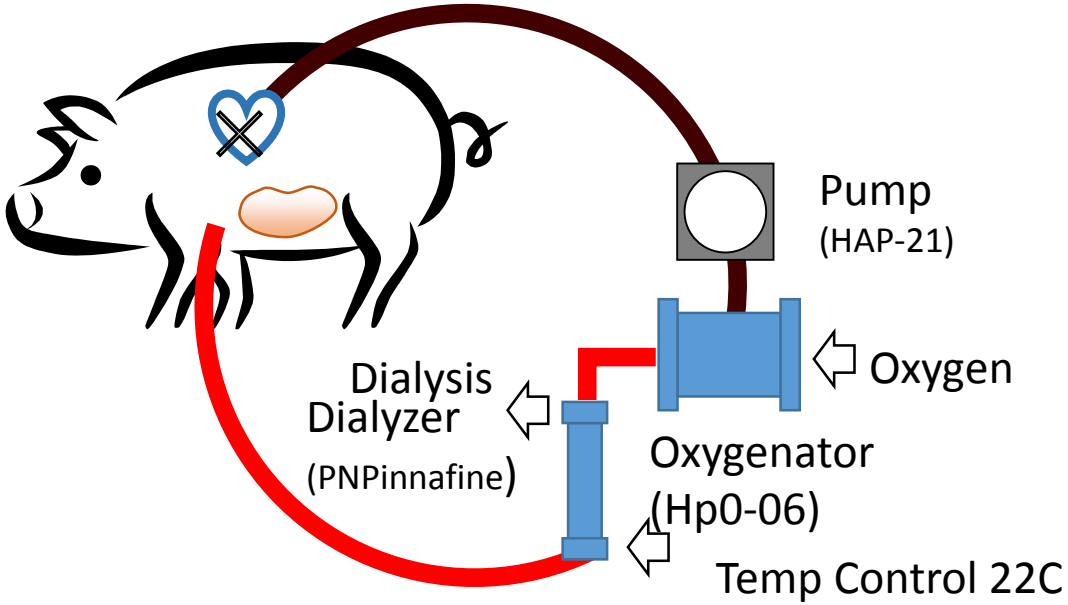
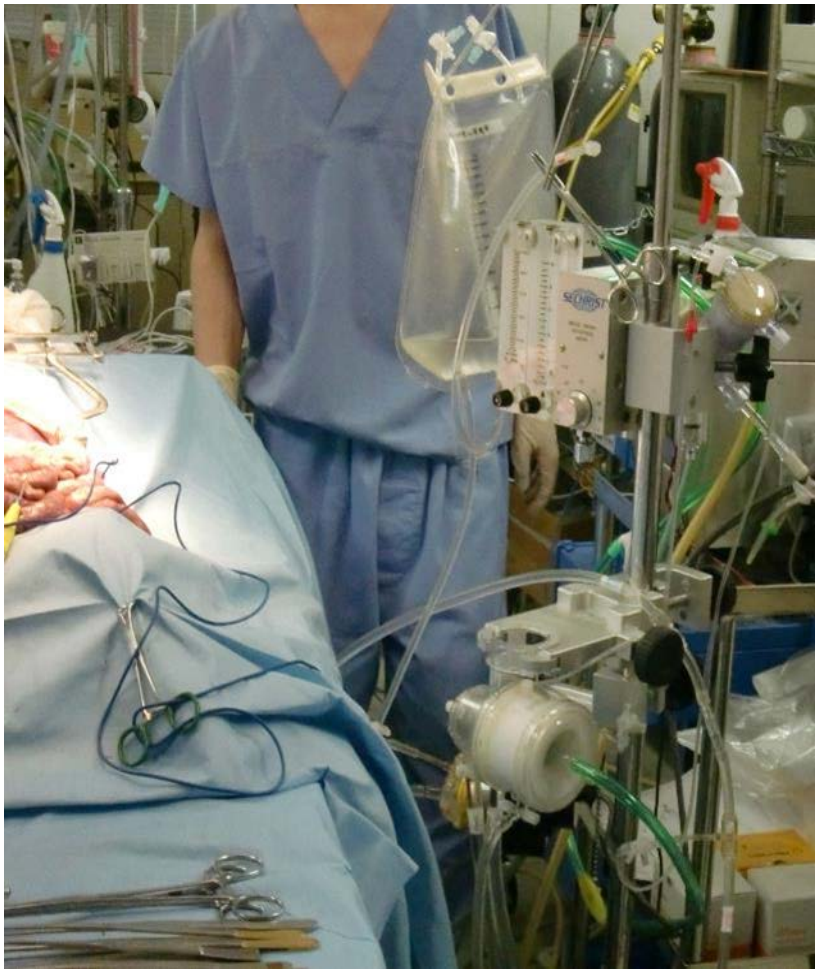

Pump
(HAP-21)
Oxygen
Dialysis
Dialyzer
(PNPinnafine)
Oxygenator
(Hp0-06)
Temp Control 22C

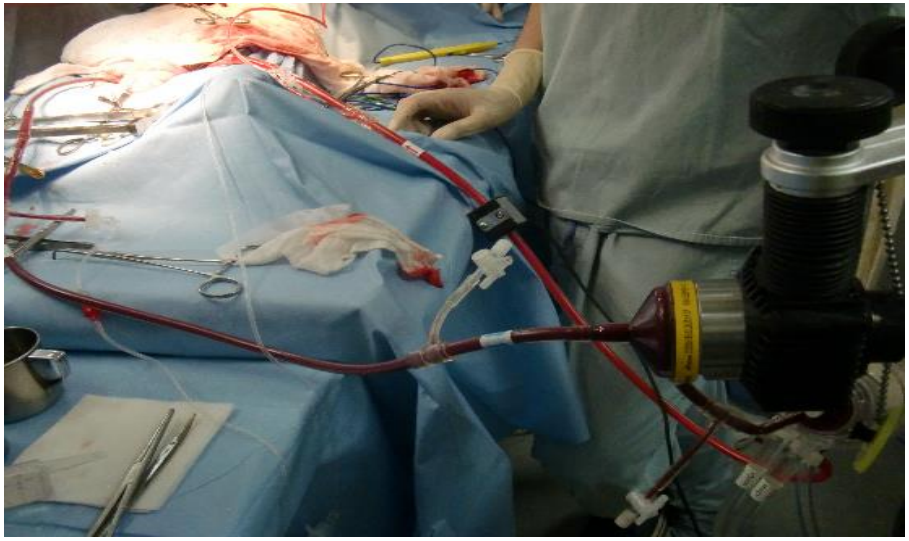
Figure1

Figure 2－A, B

A



B



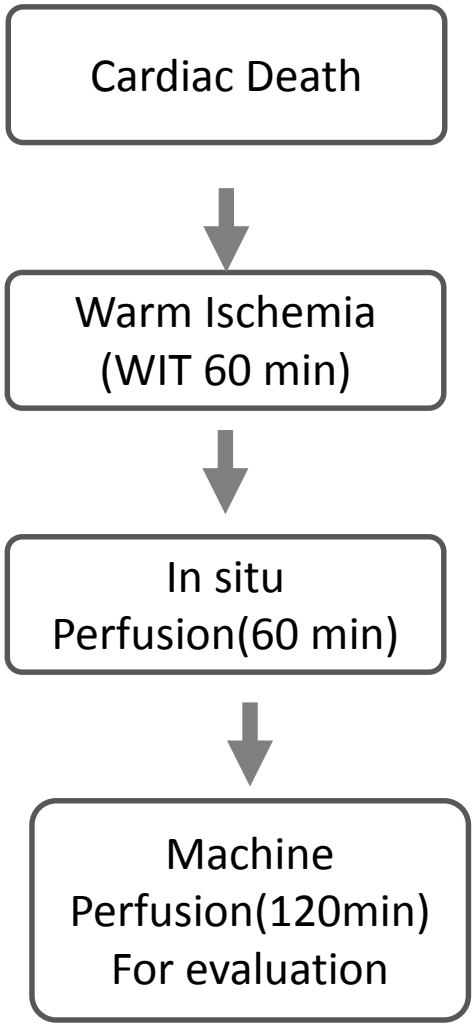
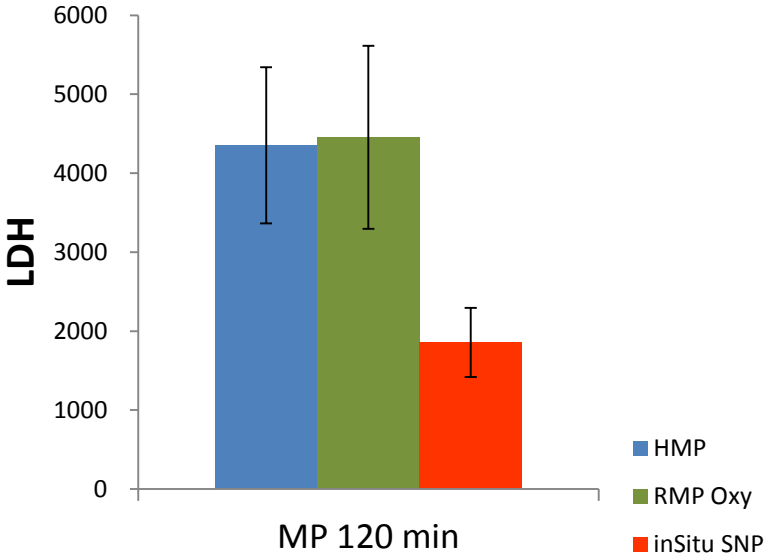

Cardiac Death
Warm Ischemia
(WIT 60 min)
In situ
Perfusion(60 min)
Machine
Perfusion(120min)
For evaluation

Figure 3


LDH
6000
5000
4000
3000
2000
1000
0
MP 120 min
HMP
RMP Oxy
inSitu SNP

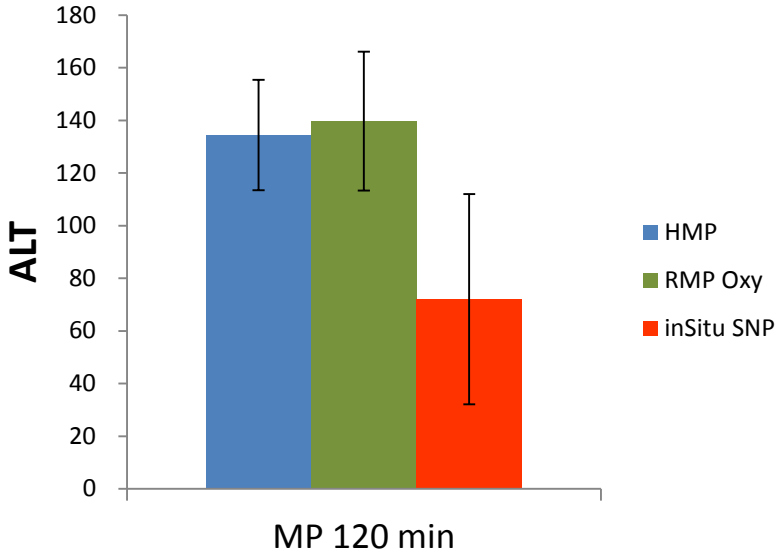

ALT
180
160
140
120
100
80
60
40
20
0
MP 120 min
HMP
RMP Oxy
inSitu SNP

Figure4

Figure 5