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Comparison of normothermic and hypothermic perfusion in porcine kidneys donated after cardiac death

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ABSTRACT

Background: Normothermic machine perfusion (NMP) is an alternative strategy for preserving kidneys donated after cardiac death (DCD). The relative efficacy of prolonged NMP compared to hypothermic machine perfusion (HMP) in DCD kidneys with moderate ischemic injury is undetermined. This study compares NMP and HMP kidney preservation in a porcine DCD model.

Methods: Ten porcine kidneys underwent NMP or HMP preservation following 45 minutes of warm ischemia and 5 hours of cold ischemia. After 8 hours of machine preservation, hemodynamic stability, renal function, perfusate biomarkers, and histologic integrity were assessed in a simulated reperfusion model.

Results: During simulated reperfusion, no differences were observed in oxygen consumption, urine production, creatinine clearance, fractional excretion of sodium, proteinuria, and perfusate levels of lactate dehydrogenase and aspartate aminotransferase. Resistance was no different after 30 minutes of simulated reperfusion. Histologically, NMP kidneys demonstrated increased vacuolization after preservation and greater loss of tubular integrity after simulated reperfusion. Perfusate levels of alkaline phosphatase (AP) and gamma glutamyltransferase (GGT) were higher in NMP kidneys during preservation, but upon simulated reperfusion, AP and GGT levels were higher in HMP-preserved kidneys. Peak AP and GGT during simulated reperfusion of HMP kidneys were over 14 times higher than peak AP and GGT during preservation of NMP kidneys.

Conclusions: NMP provided comparable preservation of renal function as HMP and minimized AP and GGT release upon reperfusion.

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Introduction

The number of patients requiring kidney transplantation is growing faster than the supply of available organs.[1] Efforts to compensate for this shortfall have led to a greater reliance on marginal quality grafts, including organs donated after cardiac death (DCD).[1,2] Such grafts are more frequently discarded,[3] and recipients experience higher rates of delayed graft function compared to kidneys donated after brain death (DBD).[4] New graft preservation modalities beyond the clinical standards of static cold storage (SCS) and hypothermic machine perfusion (HMP) could optimize utilization and outcomes of these marginal organs.

Normothermic machine perfusion (NMP) is an organ preservation technology that sustains organ grafts at physiologic temperatures. In the first clinical trial of kidney NMP, extended criteria donor kidneys that underwent 1 hour of NMP after a period of SCS had a reduced rate of delayed graft function compared to kidneys preserved with SCS alone.[5] Extending the duration of NMP offers certain advantages including minimizing ischemic and hypothermic exposure and enabling physiologic organ assessment and pharmacologic interventions.

Prolonged NMP has been attempted previously. Metcalfe *et al.* preserved DCD porcine kidneys using NMP for 16 hours following a short 8-minute period of warm ischemia.[6] Post-preservation renal function was comparable to HMP-preserved controls. More recently, Kaths *et al.* demonstrated the feasibility of 10 hours of kidney NMP in DBD porcine kidneys.[7] Subsequently, this NMP model was compared to SCS in DCD porcine kidneys after 30 min of warm ischemia.[8]

For marginal quality DCD kidneys, the relative efficacy of prolonged NMP compared to HMP is unknown. This is an important comparison since the Eurotransplant Machine Perfusion Trial showed that HMP provided superior preservation compared to SCS in DCD kidneys,[9] although a similar trial in the United Kingdom found no difference between these two modalities.[10] To address this knowledge gap, we designed a study to compare NMP and HMP kidney preservation in a porcine DCD model. Our hypothesis was that NMP-preserved kidneys would demonstrate lower vascular resistance, better renal function, and less histologic damage in a simulated reperfusion model.

Methods

Kidney procurement

Kidneys ($n = 10$) and autologous blood of domestic pigs were obtained from local slaughterhouses, which were practicing in accordance to governing regulations.[11] As previously described in translational experiments,[12-15] animals were rendered unconscious and killed by exsanguination through a carotid artery and jugular vein incision, thus initiating the warm ischemic period. Two liters of blood was collected in a heparinized container (60,000 IU), transferred to citrated blood bags, and stored on ice.

Kidney pairs were provided to researchers en bloc approximately 15 minutes after exsanguination. The left or right kidney was selected randomly for preservation, and the contralateral kidney was discarded. Gerota's fascia and perinephric fat were removed, and the renal artery, renal vein, and ureter were isolated and cannulated. The kidney was placed within an organ isolation bag. After 45 minutes of warm ischemia, the kidney was flushed through the renal artery cannula from an arterial pressure of 100 cm H_2O with 340 mL of 4°C histidine-tryptophan-ketoglutarate solution (Essential Pharmaceuticals LLC, Newtown, PA) supplemented with 2000 IU/L of heparin. Kidneys were stored in a cooler on ice for transport. After 5 hours of SCS, kidneys were assigned to either the NMP group ($n = 5$) or HMP group ($n = 5$) for a preservation time of 8 h.[8,16]

Preservation phase

Kidneys were preserved during both NMP and HMP with a perfusion system consisting of an organ chamber, a roller pump (Sarns 8000 Roller Pump, Terumo, Somerset, NJ, USA), and an oxygenator/heat exchanger (Affinity, Medtronic, Minneapolis, MN, USA), all connected by tubing (Fig. 1). Sampling ports were included before (venous) and after (arterial) the oxygenator/heat exchanger. An infusion line for supplemental drugs was connected to venous tubing. Ureteral outflow drained directly into the perfusate reservoir except when temporarily diverted into an external collection container for sampling purposes.

The NMP perfusate was a modification of the perfusate employed by Kaths *et al.*[7] It consisted of 22.5 g of bovine serum albumin, 322 mL of Krebs-Henseleit Buffer, 282 mL of lactated Ringer's solution, 44 mL of H_2O, and 202 mL of washed red blood cells (Sequestra 1000, Medtronic). The circuit was primed with perfusate, which was supplemented with 1 g of ampicillin, 1 g of cefotaxime, 2000 IU of heparin, 10 mg of dexamethasone, and 3 mL of calcium gluconate (10%). The perfusate was infused with 500 IU/h of heparin, 5 U/h of insulin lispro, 0.05 g/h of amino acids, and 0.25 mg/h of verapamil. The target mean arterial pressure was 40 mm Hg.[17] The oxygenator was supplied with 95% oxygen and 5% carbon dioxide at an initial flow rate of 0.3 L/min. To achieve a pH of 7.3-7.5, sodium bicarbonate was supplemented and the gas flow rate was adjusted as needed. A heater was set to achieve a perfusate temperature of 37°C.[7]

HMP was performed at 4°C in a cold room, and no gas was supplied.[18,19] The circuit was primed with Belzer's Machine Perfusion Solution (Bridge to Life Ltd, Columbia, SC, USA) and supplemented with 750 mg of cefuroxime, 2000 IU of heparin, 10 mg of dexamethasone, and 40 U of insulin lispro. The target mean arterial pressure was 30 mm Hg.

Simulated reperfusion phase

Following preservation, all kidneys were flushed with 150 mL of histidine-tryptophan-ketoglutarate solution at 4°C, and after a 5-minute room temperature exposure, kidneys were transferred to a secondary circuit for a 2-hour reperfusion phase to simulate transplantation.[20] This circuit contained