Normothermic Ex Vivo Kidney Perfusion Improves Early DCD Graft Function Compared With Hypothermic Machine Perfusion and Static Cold Storage

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Background. Better preservation strategies for the storage of donation after circulatory death grafts are essential to improve graft function and to increase the kidney donor pool. We compared continuous normothermic ex vivo kidney perfusion (NEVKP) with hypothermic anoxic machine perfusion (HAMP) and static cold storage (SCS) in a porcine kidney autotransplantation model. **Methods.** Porcine kidneys were exposed to 30 minutes of warm ischemia and then reimplanted following either 16 hours of either SCS, HAMP (LifePort 1.0), or NEVKP before autotransplantation (n = 5 per group). The contralateral kidney was removed. Animals were followed for 8 days. **Results.** Grafts preserved by NEVKP demonstrated improved function with more rapid recovery compared with HAMP and SCS (mean peak serum creatinine: 3.66 ± 1.33 mg/dL [postoperative d 1 [(POD1)], 8.82 ± 3.17 mg/dL [POD2], and 12.90 ± 2.19 mg/dL [POD3], respectively). The NEVKP group demonstrated significantly increased creatinine clearance calculated on POD3 (63.6 ± 19.0 mL/min) compared with HAMP (13.5 ± 10.3 mL/min, $P = 0.001$) and SCS (4.0 ± 2.6 mL/min, $P = 0.001$). Histopathologic injury scores on POD8 were lower in both perfused groups (NEVKP and HAMP, score: 1–1.5) compared with SCS (score: 1–3, $P = 0.3$), without reaching statistical significance. **Conclusions.** NEVKP storage significantly improved early kidney function compared with both cold preservation strategies, although HAMP also demonstrates improvement over SCS. NEVKP may represent a novel, superior preservation option for donation after circulatory death renal grafts compared with conventional hypothermic methods.

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INTRODUCTION

The ongoing organ shortage has generated interest in expanding the donor pool with grafts recovered from extended criteria donors or donation after circulatory death (DCD) donors. DCD kidney transplantation has increased over the past decade and represents 19.8% of kidney transplantation in the United Network for Organ Sharing region in 2017.[1] However, recipients of these

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higher-risk grafts more often experience delayed graft function (DGF) or primary nonfunction because of poorer ischemia tolerance of the organ.[2-4] Therefore, strategies to reduce preservation injury are of intense interest. Hypothermic anoxic machine perfusion (HAMP) preservation is already widely used in the clinical setting due to its simplicity. It has been demonstrated to better preserve marginal and DCD grafts compared with static cold storage (SCS). A recent comprehensive systematic meta-analysis indicated that use of HAMP resulted in lower rates of DGF in kidney transplants using all types of deceased donors (standard criteria donor, extended criteria donors, DCD) versus SCS.[5] However, HAMP was least effective in the DCD subset. Normothermic ex vivo kidney perfusion (NEVKP) is a novel technology that has been developed and extensively tested in experimental settings showing its superiority over SCS.[6] The efficacy of prolonged NEVKP compared with HAMP is less well studied and convincing data about its superiority are lacking. Our study is the first to evaluate the impact of prolonged cold and warm perfusion as preservation techniques on early graft function and markers of injury using a porcine kidney DCD autotransplantation model.

MATERIALS AND METHODS

Study Design

Graft function and injury markers were assessed in a porcine model of renal DCD autotransplantation, comparing 3 preservation methods: 16-hour SCS group, 16-hour NEVKP group, and 16-hour HAMP group. DCD conditions were mimicked by clamping the right renal artery and vein for 30 minutes in situ. The right kidney was then removed, and the vessels were cannulated. This was followed immediately by a flush with 300 mL heparinized histidine-tryptophan-ketoglutarate (HTK) before storing in ice-cold HTK solution or subjecting to warm or cold perfusion. After preservation, kidneys were again flushed with HTK before autotransplantation. The contralateral kidney was removed before donor implantation. Pigs were followed for 8 days after transplantation and then humanely euthanized for the collection of histology samples. The study protocol was approved by the Animal Care Committee of the Toronto General Research Institute, Ontario, Canada (Figure 1).

Animals and Surgical Protocol

Three-month-old male Yorkshire pigs (≈30 kg) were housed for 1 week before experimentation with water and food provided ad libitum. All animal procedures and care were regulated according to the Principles of Laboratory Animal Care by the National Society for Medical Research and the Guide for the Care of Laboratory Animals published by the National Institutes of Health.

The surgical and anesthetic procedures were conducted as previously described elsewhere by our group.[7] Briefly, animals were premedicated with intramuscular injections of ketamine (20 mg/kg; Bimeda-MTC Animal Health Inc., Cambridge, Canada), atropine (0.04 mg/kg; Rafter 8 Products, Calgary, Canada), and midazolam (0.3 mg/kg; Pharmaceutical Partners of Canada Inc., Richmond Hill, Canada). Then general anesthesia was induced and maintained by administration of inhaled isoflurane (1.5%; Pharmaceutical Partners of Canada Inc., Richmond Hill, Canada) and continuous intravenous administration of Propofol (150 mg/h; PharmaScience Inc., Montreal, Canada). Pigs were intubated and a central venous catheter (9.5F; Cook Medical Company, Bloomington) was inserted into the internal jugular vein for administration of fluids and medications. An abdominal approach with a midline incision was used to access the right kidney. The renal artery and vein were dissected and clamped with vascular pedicles for 30 minutes before the removal of the kidney. At the back table, the renal vessels were then cannulated and the kidney was flushed with 300 mL HTK, containing 10 000 IU/L heparin (Sandoz Canada Inc., Toronto, Canada) and attached to the warm or cold perfusion device or stored in ice-cold HTK. The abdomen was closed, and the pig was recovered until transplantation. At the end of the preservation period, the pig was reanesthetized for autografting. The midline wound was reopened, and a contralateral nephrectomy was performed. Then the donor graft was disconnected from the perfusion pump or removed from ice and reflushed with 4°C HTK (300 mL) and kept cold until the renal anastomosis (vein: end-to-side to inferior vena cava, artery: end-to-side to aorta, ureter: side-to-side) was sewn. Perioperative procedures, drug administration, and care of the pigs were conducted as previously described.[7]

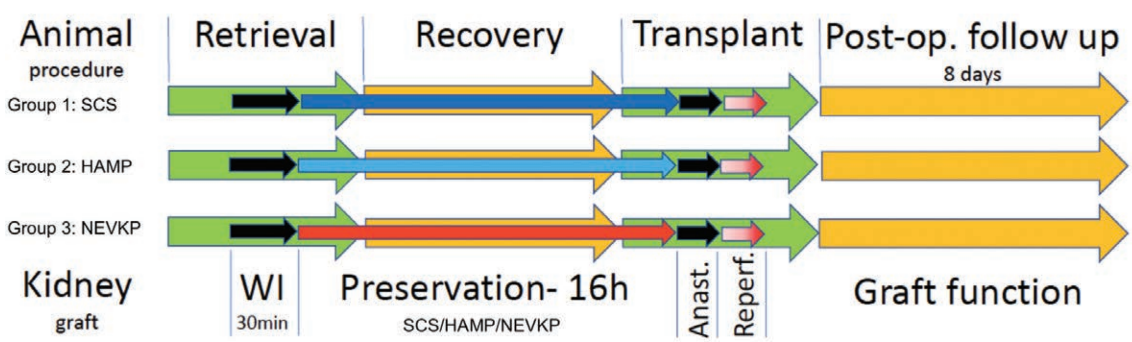

FIGURE 1. Study design. Pigs were randomly assigned into 3 groups (n: 5 each), kidneys were retrieved after 30 min warm ischemia and preserved by 16 h of SCS, HAMP, or NEVKP. Then they were autografted into the same pig with contralateral kidneys removed. The animals were followed for 8 days and sacrificed for histology assessment. HAMP, hypothermic anoxic machine perfusion; NEVKP, normothermic ex vivo kidney perfusion; SCS, static cold storage.

Static Cold Storage

After flushing, the kidneys were placed in a sterile organ bag (CardioMed Supplies Inc., Lindsay, Canada) filled with HTK solution and stored at 4°C in an icebox for 16 hours.

Hypothermic Anoxic Machine Perfusion

The LifePort 1.0 device (Organ Recovery Systems, Itaska, IL) and circuit were prepared sterilely according to the manufacturer's instructions. One liter of Belzer Machine Perfusion Solution (Bridge to Life Ltd, Columbia, SC) was used as a perfusate. After flushing with 4°C cold heparinized HTK (300 mL), the renal artery was cannulated with a 3 mm straight cannula (Organ Recovery Systems, Itaska, IL) and tied with 2-0 silk ties (Covidien, Mississauga, Canada). After priming the circuit, the kidney was placed into the cassette and perfused with a mean arterial pressure of 30 mm Hg. The temperature was maintained between 3°C and 5°C throughout the preservation. The perfusate was sampled every 2 hours and stored at −80°C for further analysis. After the cold perfusion preservation, the kidney was removed and reflushed with HTK (300 mL) and stored on ice until the anastomosis was initiated.

Normothermic Ex Vivo Kidney Perfusion

Our pressure controlled, nonpulsatile NEVKP system was designed using a pediatric cardiopulmonary bypass device as described previously.[8] Briefly, the S3 heart-lung machine and the circuit consisted of a centrifugal pump (Sorin Group Inc., Markham, Canada), an oxygenator (Sorin Group Inc., Markham, Canada), a venous reservoir, an arterial bubble filter (D130 neonatal arterial filter, Sorin Group Inc.), and polyvinyl chloride tubing. Additionally, a heat exchanger, syringe and infusion pumps for medications and fluid replacement, a flow transducer, a temperature probe, and a customized double-walled heated organ chamber were built into the system. Perfusion circuit parameters (temperature, arterial and venous pressure, and arterial flow) were continuously recorded by using the Data Management System (Sorin Group Inc.).

The perfusion circuit was primed with Ringer lactate (200 mL), STEEN Solution (150 mL; XVIVO Perfusion AB, Goteborg, Sweden), washed leukocyte-filtered erythrocytes (125 mL), 27 mL of double reverse osmosis water, 8 mL sodium bicarbonate (8.4%), and 1.8 mL calcium gluconate (10%), resulting in a near physiologic electrolyte composition, osmolality, oncotic pressures, and hemoglobin level of approximately 90 g/L. Heparin was added to the perfusate for anticoagulation as not all platelets and clotting factors were removed.

Oxygen/carbon dioxide gas (95%/5%; 2 L/min) and verapamil (0.25 mg/h) were administered continuously during the perfusion. An infusion of amino acids, glucose, and insulin (Dextrose + Travasol + Humulin R, 1 mL/h) was also provided and adjusted to maintain perfusate glucose level between 5 and 15 mmol/L. Ringer lactate solution (10 mL/h) was used to replace urine output and to account for other insensible losses from the system.

The renal artery, renal vein, and ureter were cannulated (1.6″ cannula, Sorin Group Inc., 1/4 × 1/8″ cannula Sorin Group Inc., and pediatric feeding tube, respectively) and fixed with 2-0 silk ties (Covidien, Mississauga, Canada) before connecting to the previously primed circuit. Initial arterial pressure was set at 75 mm Hg as previously described and maintained at 65 mm Hg by adjusting the rate of the centrifugal pump. The venous pressure was maintained between 4 and 5 mm Hg.

Urine and perfusate samples were collected hourly, centrifuged at 9000 rpm for 10 minutes, and stored at −80°C for further investigation. Blood gas analysis (RAPID Point 500 Systems; Siemens AG, Berlin, Germany) of the perfusate was performed before storage of the sample.

Whole Blood, Serum, and Urine Measurements

Blood gas analysis (RAPID Point 500 Systems; Siemens AG, Berlin, Germany) was used for blood samples taken with a heparinized syringe from the previously placed central venous catheter. After centrifugation at 9000 rpm for 10 minutes, serum samples were also analyzed for creatinine and aspartate aminotransferase (AST) using a point-of-care comprehensive metabolic blood chemistry analyzer (Piccolo Xpress, Union City, Canada) before the transplant and daily during the postoperative course. Twenty-four–hour urine collection was measured before transplantation, at postoperative day (POD)2–3, and at POD7–8 using a customized metabolic cage. Twenty-four–urine collection, urine obtained by bladder puncture before transplantation and at time of sacrifice, and daily serum samples were sent to the Toronto General Hospital Core Laboratory for analysis with the Abbott Architect Chemistry Analyzer using the manufacturer's reagents (Abbott Laboratories, Abbott Park, IL). Porcine neutrophil gelatinase-associated lipocalin (NGAL) ELISA kit (Bioporto, Hellerup, Denmark) was used for serum samples according to the manufacturer's instructions.

Histology

Baseline needle biopsies were taken from the contralateral kidney during the retrieval. Under deep anesthesia at the time of sacrifice on POD8, wedge biopsies were taken from the renal graft. Tissue was placed in 10% neutral buffered formalin and transferred to 70% alcohol after 48 hours. Following paraffin-embedding, sectioning (3 μm), and periodic acid-Schiff (PAS) staining, sections were used to score global tubular injury and interstitial inflammation on a semiquantitative scale of 0–3 by a blinded renal pathologist. The tubular injury score was based on degree of brush border loss, tubular dilatation, epithelial vacuolation, thinning and sloughing, and luminal debris and averaged over 10 high power fields. Interstitial inflammation was scored in 10 low power fields and averaged. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed according to standard protocol and analyzed by Aperio ImageScope software.

Statistical Analysis

SPSS software version 22.0 (IBM, Armonk, NY) was used for statistical analysis. Values are presented as means ± SD. For multiple comparisons, 1-way ANOVA was used with Tukey honestly significant difference post hoc test to identify significant differences between the selected groups. The Kruskal-Wallis test was used for comparison of nonparametric variables. A paired T-test was used to test significance of differences in normally distributed continuous parameters over time within the same group. Significance was defined as $P < 0.05$.

RESULTS

Animal Demographics

There was no difference in average animal weight (SCS: 31.6 ± 3.2 kg, NEVKP: 30.2 ± 2.4 kg, HAMP: 30.8 ± 1.2 kg, $P = 0.80$) between groups. All animals survived until day 8 in each group except 1 in the HAMP group. This pig was euthanized on day 7 because of sudden respiratory distress, although its renal function, as demonstrated by serum creatinine, was comparable to other animals in this group. The postmortem pathology findings showed acute respiratory failure with mild pulmonary infiltration of unclear etiology.

Hypothermic Machine Perfusion

Kidney grafts that were subjected to cold perfusion demonstrated improvement in flow rates (baseline: 16.0 ± 6.1, 1 h: 45.2 ± 6.7, 15 h: 53.6 ± 11.7 mL/min) and resistance index (baseline: 1.70 ± 0.17, 1 h: 0.59 ± 0.12, 15 h: 0.45 ± 0.09 mm Hg·min/mL) during preservation, with a significant difference between values at baseline compared with at 1 hour ($P < 0.001$). The temperature was maintained between 3°C and 5°C throughout the perfusion. A minimal decrease in perfusate glucose concentration was observed (baseline: 10.2 ± 0.4 mmol/L, 15 h: 9.3 ± 0.5 mmol/L, $P = 0.053$) along with a significant increase in lactate concentration (baseline: 0.28 ± 0.07 mmol/L, 15 h: 0.88 ± 0.13 mmol/L, $P = 0.0033$) during perfusion (Figure 2).

NEVKP: Perfusion Characteristics and Viability Markers

Renal blood flow progressively increased during the perfusion (baseline: 62 ± 19 mL/min, 16 h: 215 ± 30 mL/min, $P < 0.01$), whereas the intrarenal vascular resistance significantly decreased (baseline: 1.7 ± 0.2 mm Hg·min/mL, 15 h: 0.45 ± 0.09 mm Hg·min/mL, $P < 0.001$). The acid-base parameters and electrolyte concentrations (pH, bicarbonate, base excess, serum sodium, potassium, calcium, and chloride) were stable within physiologic range during the entire time of NEVKP (data not shown). Rapid lactate clearance was observed (baseline: 9.6 ± 0.6 versus 3 h: 2.3 ± 1.0 mmol/L, $P < 0.01$) and remained low throughout perfusion (16 h: 1.6 ± 0.6 mmol/L). All kidneys produced urine during the perfusion. Enzyme markers of cellular injury, such as AST and lactate dehydrogenase (LDH), increased during first half of the perfusion, peaked at 7 hours, and stabilized until the end of the perfusion from the baseline value (AST baseline: 1.0 ± 0.4, 7 h: 31.8 ± 10.0, 16 h: 27 ± 15 U/L, $P < 0.05$ for 1 h versus 16 h, LDH baseline: 1 ± 0, 7 h: 37 ± 26, 16 h: 32 ± 21 U/L, $P < 0.05$ for 1 versus 16 h) (Figure 3).

Posttransplant Graft Function

Kidneys preserved by warm perfusion demonstrated improved graft function and faster recovery with lower mean peak serum creatinine that occurred earlier compared with HAMP and SCS (POD1: 3.66 ± 1.33 mg/dL, versus POD2: 8.82 ± 3.17 mg/dL, and POD3: 12.90 ± 2.19 mg/dL, respectively) (Figure 4). The differences between daily serum creatinine levels reached significance between NEVKP and HAMP on days 1, 2, and 3 ($P = 0.002$, $P = 0.004$, $P = 0.024$) and between HAMP and SCS on days 3 and 4 ($P = 0.016, 0.046$). POD3 creatinine clearance was significantly impaired in both HAMP and SCS groups compared with NEVKP (13.5 ± 10.3 and 4.0 ± 2.6 mL/min, versus 63.6 ± 19.0 mL/min, respectively, $P = 0.001$). Fractional excretion of sodium (FENA) measured on POD3 as a marker of tubular function was significantly lower in the NEVKP group compared with SCS group (0.48 ± 0.34% versus 19.6 ± 18.5%, $P = 0.033$). FENA also trended toward lower values in the NEVKP group compared with the HAMP group, although significance was not reached (0.48 ± 0.34% versus 3.11 ± 1.80%, $P = 0.067$) (Figure 5).

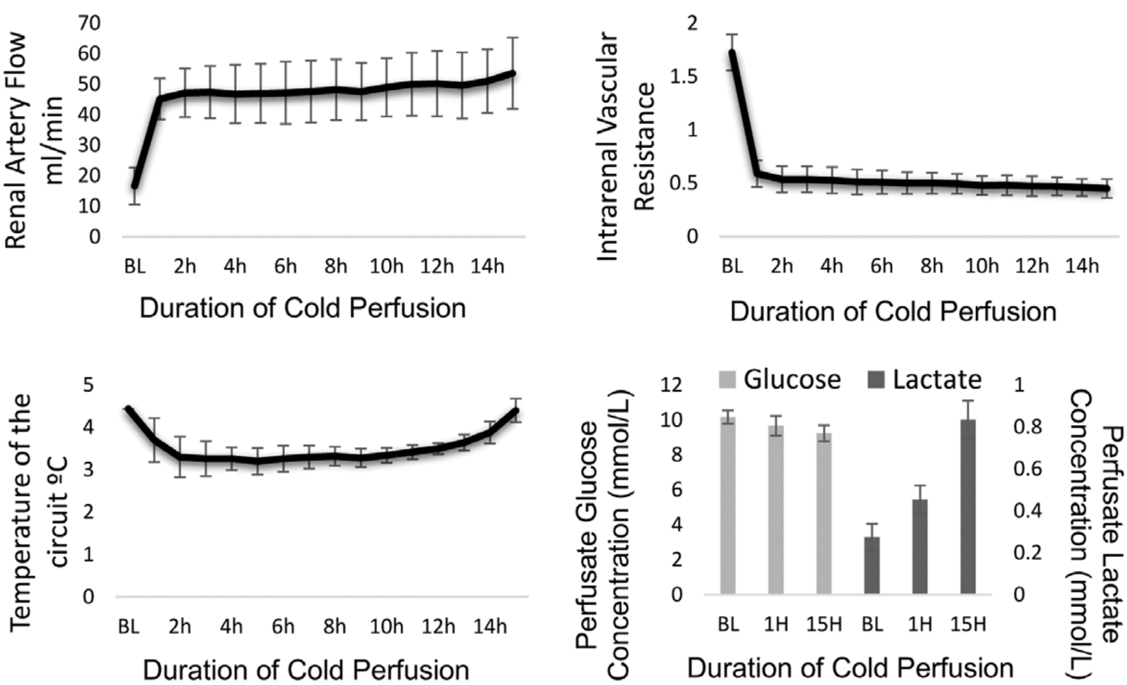

FIGURE 2. Pump parameters (flow, resistance, temperature) and perfusate metabolite (glucose and lactate) levels during cold machine perfusion. The values are presented as mean ± SD.

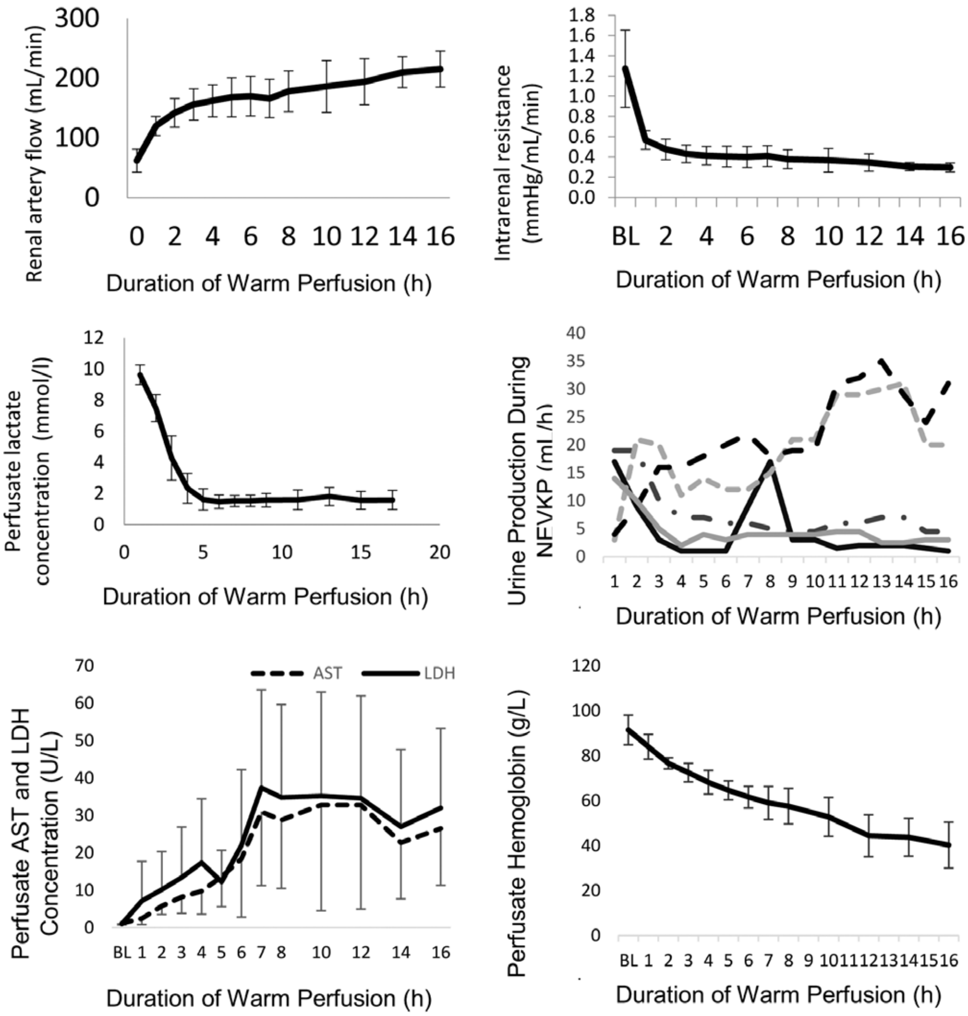

FIGURE 3. Pump parameters (flow, resistance), functional markers (lactate clearance, urine production), cellular injury markers (aspartate aminotransferase [AST], lactate dehydrogenase [LDH]), and perfusate hemoglobin level during normothermic ex vivo kidney perfusion (NEVKP). The values are presented as mean ± SD. Urine production is presented as individual experiments with different dashed lines representing individual experiments.

Posttransplant Kidney Injury and Inflammation

Renal tissue samples were collected for histology at time of sacrifice on POD8. Periodic acid-Schiff–stained slides were then assessed for tubular injury and inflammation in a semiquantitative scale evaluated by a blinded renal pathologist. Tubular injury was equally low (score, 1–1.5) in the perfused groups (NEVKP, HAMP) and highest in the SCS group (score, 1–3); however, the difference did not reach statistical significance (P = 0.3) (Table 1). TUNEL staining showed significantly more positive cells in the SCS group compared with the perfused groups (versus NEVKP: P = 0.005, versus HAMP: P = 0.018 (Figure 6). Postoperative serum NGAL was measured on POD2 when serum creatinine began to diverge among all groups and was elevated in SCS compared with the perfused groups (P = 0.02) (Figure 6).

DISCUSSION

Our laboratory has previously demonstrated that 16 hours of NEVKP results in improved graft function over SCS in our porcine 30 minutes DCD renal autotransplantation model[9,10]; however, graft function following HAMP was not previously compared with these 2 conditions.[11,12] To our knowledge, this study is the first to assess this question with a porcine DCD autotransplantation model. Sixteen hours of storage was utilized to mimic relevant storage times encountered clinically. Eight days of follow-up was completed to allow for the assessment of the entire serum creatinine curves of all groups. We demonstrated that both NEVKP and HAMP provided protection against graft injury compared with SCS. However, kidneys subjected to NEVKP demonstrated even greater protection from injury and faster functional recovery than those subjected to HAMP. NEVKP resulted in more rapid recovery of renal and tubular function as demonstrated through postoperative serum creatinine, creatinine clearance, and fractional secretion of sodium. In contrast, early graft function was impaired in the both cold storage strategies, with static cold stored kidneys demonstrating significant TUNEL positivity and elevated POD2 serum NGAL levels. Together, this likely indicates an evolving process of injury in the cold control groups from which the warm perfused kidney was protected.

Before this study, only a few research groups compared the impact of prolonged cold and warm perfusion

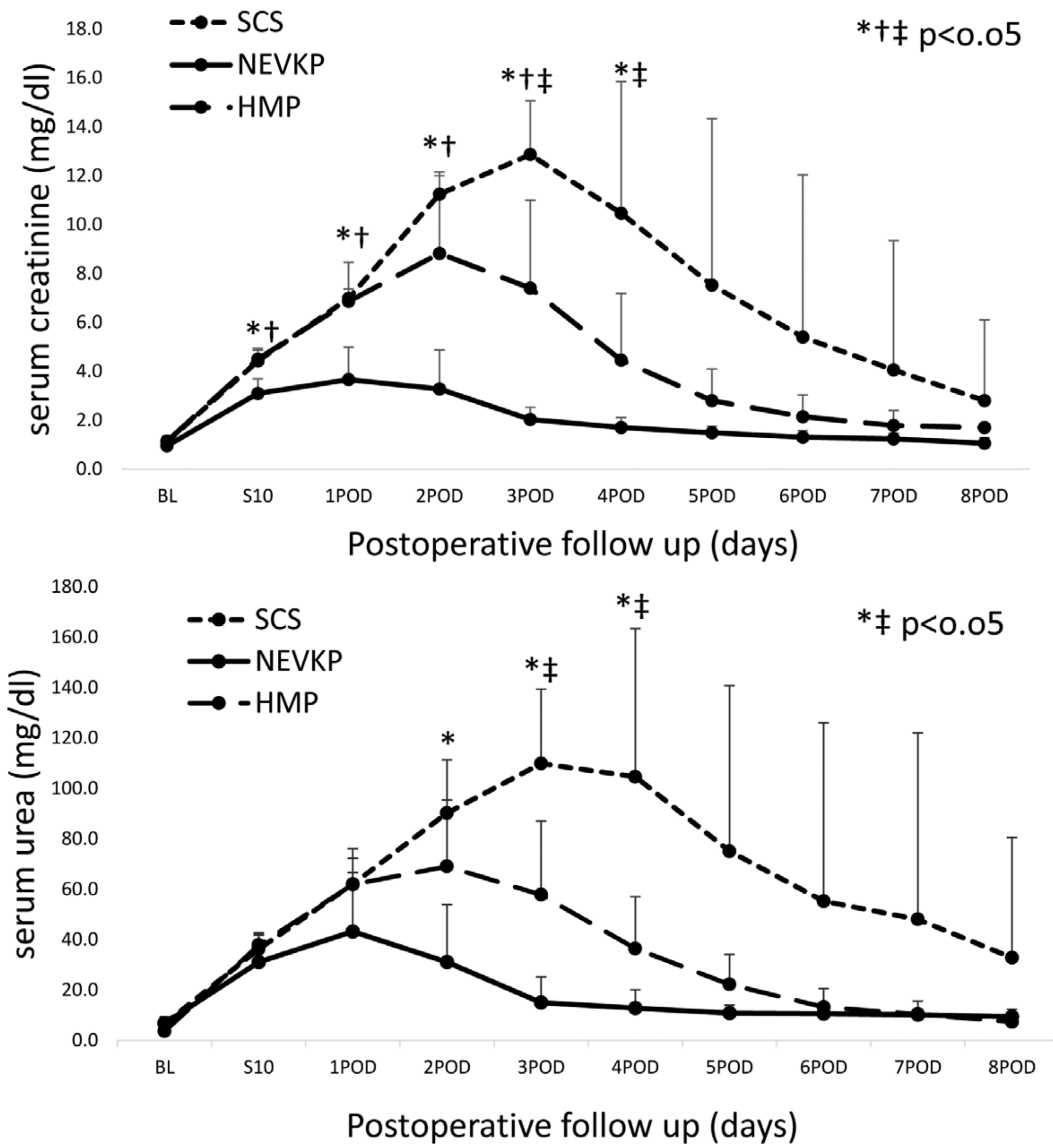

FIGURE 4. Graft function. Postoperative serum creatinine and urea levels. The values are presented as mean ± SD. *Significance between normothermic ex vivo kidney perfusion (NEVKP) and static cold storage (SCS), †significance between NEVKP and hyothermic anoxic machine perfusion (HMP), ‡significance between HMP and SCS, $P < 0.05$. BL, baseline; POD, postoperative day; SD, standard deviation; S10, 10 h after reperfusion.

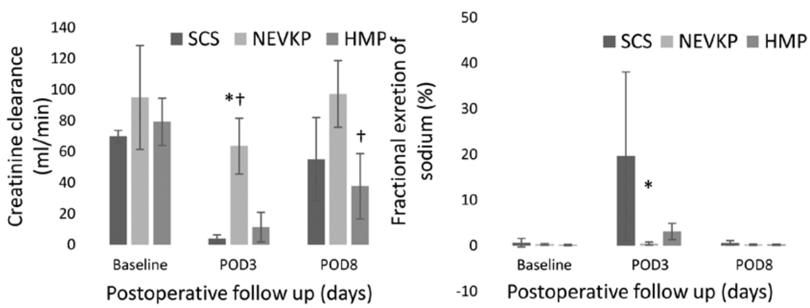

FIGURE 5. Posttransplant glomerular (creatinine clearance) and tubular function (FENA). *Significant difference between static cold storage (SCS) and normothermic ex vivo kidney perfusion (NEVKP), †significant difference between NEVKP and hypothermic anoxic machine perfusion (HMP). $P < 0.05$. FENA, fractional excretion of sodium; POD, postoperative day.

preservation. The Hosgood-Nicholson group compared paired porcine kidneys injured with 8 minutes of warm ischemia that were then subjected to normothermic autologous blood perfusion using the pulsatile organ perfusion system (Transmedics) or HAMP (Waters RM3 device) for 16 hours.[13] An ex vivo reperfusion system was

TABLE 1.
Histology scores

	Tubular injury ($P = 0.3$)				Interstitial inflammation ($P = 0.71$)			
POD8	Score 0	Score 1	Score 2	Score 3	Score 0	Score 1	Score 2	Score 3
SCS	0	2	2 (1)	1	0	3	2 (1)	0
NEVKP	0	4	1 (1)	0	0	4	1	0
HMP	0	4	1 (1)	0	2	2	1 (1)	0

Experimental groups were blinded to the assessing pathologist. This table depicts the number of specimens given a particular score within a group. (n) denotes specimens that were given a half-score by the pathologist and these were rounded upward for this analysis. None of groups differed significantly regarding injury ($P = 0.3$) or inflammation scores ($P = 0.71$).
HMP, hypothermic anoxic machine perfusion; NEVKP, normothermic ex vivo kidney perfusion; POD, postoperative day; SCS, static cold storage.

utilized for 2 hours following preservation to assess graft function. Warm perfused kidneys had significantly higher sodium reabsorption and a higher urine-to-plasma creatinine ratio, but no difference in other markers of function and injury such as creatinine clearance, vascular resistance, proteinuria, and glycosuria. The same group also investigated different preservation techniques in another study where kidneys from pigs were subjected to 10 minutes of warm ischemia and assigned into 1 of 4 conditions: 2 hours of SCS, 18 hours of SCS, 18 hours of HAMP, and 16 hours SCS plus 2 hours NEVKP.[14] Graft function was again assessed by 2 hours of ex vivo reperfusion. The 16 hours SCS plus 2 hours NEVKP group was found to have similar graft function to the 2 hours of SCS group, indicating that the 2 hours of warm perfusion time could recondition the kidney to overcome the damage from the

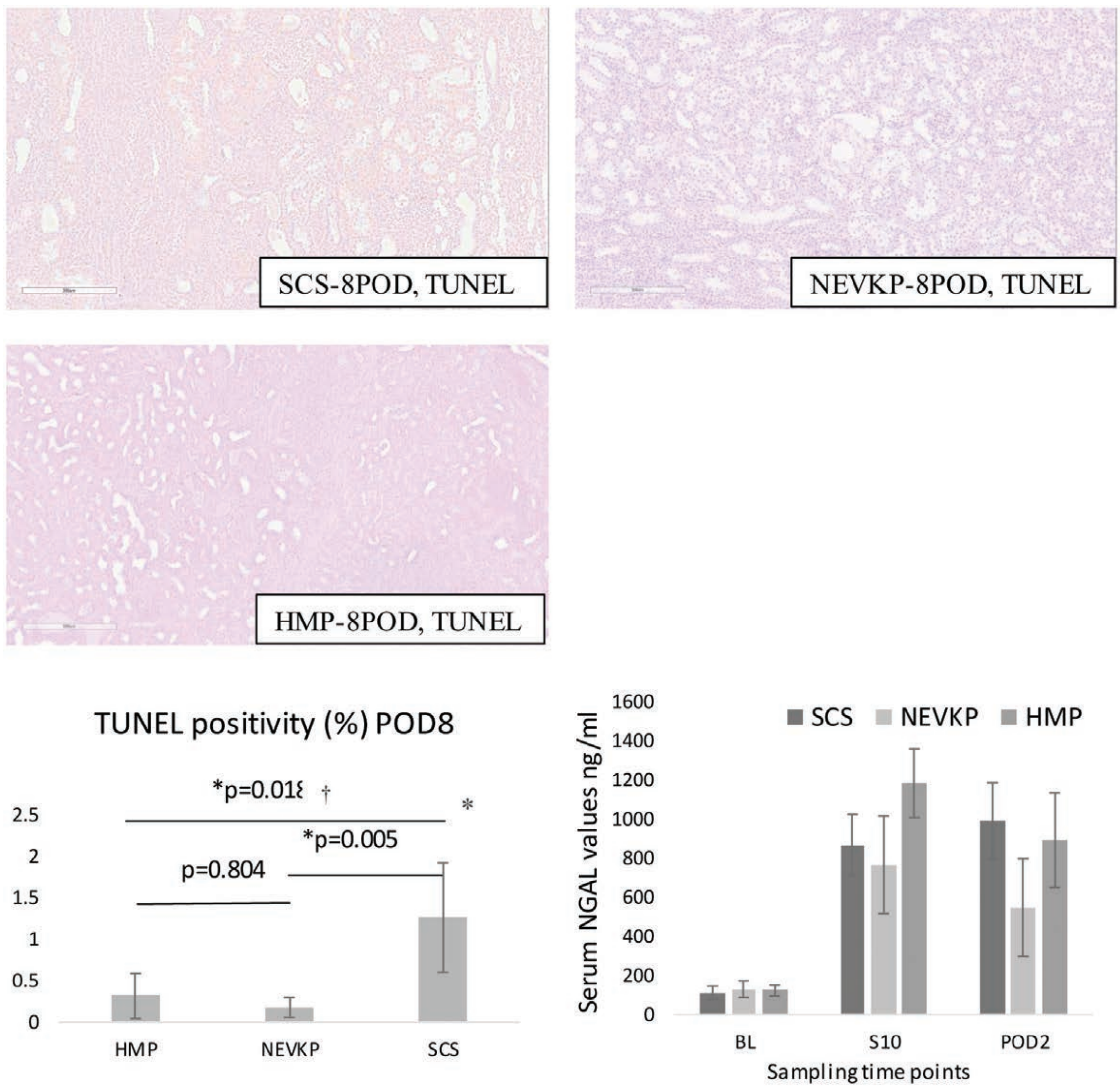

FIGURE 6. Graft injury. Representative histology images (terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL] staining), TUNEL positivity (%). Serum neutrophil gelatinase-associated lipocalin (NGAL) values. The values are presented as mean ± SD. *Significance between normothermic ex vivo kidney perfusion (NEVKP) and static cold storage (SCS), †significance between NEVKP and hypothermic anoxic machine perfusion (HMP). $P < 0.05$. POD, postoperative day.

additional prolonged SCS time in this group. The 16 hours SCS plus 2 hours NEVKP group was also equivalent to 18 hours HAMP regarding renal blood flow, creatinine clearance, FENA, urine output, histology scoring, and endothelial damage. Only minimal improvements were detected in oxygen consumption, adenosine triphosphate/adenosine diphosphate ratio, and acid-base balance in favor of the 16 hours SCS plus 2 hours NEVKP group. In contrast, the degree of cytoplasmic vacuolization and AST were significantly higher in 16 hours SCS plus 2 hours NEVKP group compared with the 3 other groups. Blum et al[15] compared function and injury of porcine kidneys treated with 45 minutes of warm ischemia followed by 8 hours of hypothermic or warm machine perfusion technology. Using a model of ex vivo reperfusion following preservation to assess graft quality, both perfusion techniques resulted in similar vascular resistance indices and renal function that included urine production, creatinine clearance, FENA, and proteinuria. Oxygen consumption, LDH, and AST release into the perfusate also did not differ between groups. Interestingly, perfusate gamma-glutamyl transferase and alkaline phosphatase levels, which may indicate proximal tubular injury, were higher in the normothermic group during perfused preservation, but the levels were reversed after ex vivo reperfusion. The authors interpreted this observation that HAMP may delay the progression of inevitable kidney injury during preservation that then became apparent once the graft was reperfused. In contrast, they proposed that NEVKP might be able to limit the extent of preexisting injury during ex vivo reperfusion.

Ex vivo reperfusion to assess graft viability and function following various storage strategies in experimental settings is frequently used because of its relative simplicity and cost compared with reimplantation of grafts in animal models; however, it has several major limitations. First, ex vivo reperfusion can only be performed for a few hours, which might be too short to determine the extent of kidney injury and function following preservation. Second, the ex vivo perfusate lacks important cellular components and mediators of injury and regeneration, including platelets, leukocytes, and cytokines. Third, ex vivo reperfusion by itself might induce injury, which could mask differences between the perfused preservation groups. Furthermore, reperfusion injury itself may not result in measurable parameters or structural changes that an ex vivo reperfusion model could identify. Therefore, the ex vivo reperfusion model is useful in pilot experiments assessing preservation techniques, but in vivo studies with transplantation of grafts following preservation are important confirmatory experiments.

To our knowledge, there are no studies that compared NEVKP with HAMP head-to-head in a clinically relevant in vivo survival model. Studies that have attempted to address this have important limitations in their experimental design. Brasile et al[16] used a canine autotransplantation model to assess graft function after preservation. Following 120 minutes of warm ischemia, kidneys were perfused at subnormothermic temperature (32°C) for 18 hours using an acellular perfusate containing enriched tissue culture-like solution and pyridoxylated bovine hemoglobin as an oxygen carrier. Control kidneys were subjected to 18 hour hypothermic machine perfusion (Waters MOX-100) before reimplantation. In the HAMP group (n:2), all animals were euthanized due to primary nonfunction and subsequent anuria. In contrast, all 5 animals survived in the subnormothermic perfusion group and had complete functional recovery with normal renal biochemistry at the end of the observation period. In a porcine autotransplant model using kidneys damaged by 30 minutes of warm ischemia, Hosgood et al[17] investigated 2 hours NEVKP reconditioning after 20 hours HMP and compared this with 22 hours HAMP. There was no significant survival or functional differences between these groups; however, lipid peroxidation as a marker of oxidative stress was lower in the group that received NEVKP. Nevertheless, neither study was able to demonstrate a clear advantage of NEVKP or HAMP.

Our study addressed this by directly comparing the storage of kidneys subjected to 30 minutes of warm ischemia with 16 hours of NEVKP, HAMP, or SCS, in a clinically relevant porcine renal autotransplantation model. During NEVKP, nearly physiologic perfusate conditions are achieved with nutrition provided to the graft at normal body temperatures. Oxygen was supraphysiologic in our set-up. The perfusate consists of normal electrolyte levels, acid-base properties, oncotic pressures, and osmolarity, which are stable for the 16 hours of perfusion. Kidneys did not show signs of deterioration during perfusion. Hemodynamic parameters including intrarenal resistance and renal blood flow progressively improved during preservation. Conversely, markers of cellular injury including AST and LDH stabilized and remained low. The lactate elimination and glucose consumption observed indicate that aerobic metabolism was being promoted, and these markers were previously correlated with improved post-transplantation graft function by our laboratory.[18]

This work also demonstrated improved DCD graft function following preservation with HAMP compared with SCS. At time of sacrifice, histology of HAMP-stored grafts had lower TUNEL positivity and trended toward improved histological scores compared with SCS-stored grafts. Lower and earlier peak serum creatinine values were also observed in HAMP-stored grafts compared with SCS. Although the improvement in serum creatinine was even greater following NEVKP preservation, HAMP devices offers a logistical advantage in its portability that NEVKP cannot currently replicate.

The mechanisms that account for the improved performance of perfusion-stored grafts over SCS were not directly addressed in this work. Perfusion itself may provide for the washout of toxic and inflammatory mediators that accumulate in DCD grafts due to anaerobic metabolism. Moreover, the flow of fluid can also provide structural integrity and protect endothelial cell surfaces. Dissolved oxygen in cold perfusates may also be sufficient to prevent further basal anaerobic metabolism in HAMP-stored grafts. Further improvements with NEVKP may be a result of oxygen in sufficient quantities at normal body temperatures to promote continued aerobic metabolism in a physiological environment. This environment may also promote repair of the graft during storage. Further studies are required to determine the precise mechanisms responsible for the improved function of perfusion-stored grafts.

Furthermore, limitations imposed by the experimental model preclude a decisive assessment of the effects of perfusion storage on DGF. The small sample size may not allow for significance to become apparent in histological findings, and important histological changes occurring before sacrifice at earlier time points that may have consequences for long-term function could not be assessed in this large animal model. The inability to follow large animals over a prolonged time also precludes assessment of long-term graft function. Currently, we can only infer a probable improvement in DGF rates in NEVKP-stored grafts over HAMP storage, which itself is improved over SCS storage, based on early postoperative serum creatinine values.

In summary, our work suggests that NEVKP should be considered for the preservation of marginal renal grafts in the clinical setting. In this study, NEVKP improved marginal graft function and promoted faster recovery compared with HAMP, which could possibly reduce DGF rates. Although no significant differences were identified in these groups with respect to histology, the late time point used for analysis as well as the relatively mild kidney injury used in this model could account for this finding. NEVKP also offers other advantages, including the possibility to directly treat and modify marginal grafts before transplantation to improve outcome. Furthermore, the viability of organs can be assessed in real time during perfusion by monitoring functional parameter, metabolic end-points, and injury. Together, this will allow for the consideration of marginal grafts that would otherwise be declined and thus potentially increase the pool of available organs for transplantation.

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