Combining Oxygenated Cold Perfusion With Normothermic Ex Vivo Perfusion Improves the Outcome of Donation After Circulatory Death Porcine Kidney Transplantation

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Background. Ex vivo machine perfusion is a novel preservation technique for storing and assessing marginal kidney grafts. All ex vivo perfusion techniques have advantages and shortcomings. The current study analyzed whether a combination of oxygenated hypothermic machine perfusion (oxHMP) followed by a short period of normothermic ex vivo kidney perfusion (NEVKP) could combine the advantages of both techniques. **Methods.** Porcine kidneys were exposed to 30 min of warm ischemia followed by perfusion. Kidneys underwent either 16-h NEVKP or 16-h oxHMP. The third group was exposed to 16-h oxHMP followed by 3-h NEVKP (oxHMP + NEVKP group). After contralateral nephrectomy, grafts were autotransplanted and animals were followed up for 8 d. **Results.** All animals survived the follow-up period. Grafts preserved by continuous NEVKP showed improved function with lower peak serum creatinine and more rapid recovery compared with the other 2 groups. Urine neutrophil gelatinase-associated lipocalin, a marker of kidney injury, was found to be significantly lowered on postoperative day 3 in the oxHMP + NEVKP group compared with the other 2 groups. **Conclusions.** A short period of NEVKP after oxHMP provides comparable short-term outcomes to prolonged NEVKP and is superior to oxHMP alone. A combination of oxHMP with end-ischemic NEVKP could be an attractive, practical strategy to combine the advantages of both preservation techniques.

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INTRODUCTION

Kidney transplantation results in improved survival and quality of life in patients with end-stage kidney disease.[1,2] However, the number of suitable donors is limited, and stringent donor criteria further restrict the pool of available kidney grafts for transplantation.[3] To overcome the

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organ shortage, grafts from extended criteria donors and donation after circulatory death (DCD) have been increasingly accepted for transplantation. These grafts tolerate anoxic cold preservation poorly, with a higher rate of postoperative delayed graft function, primary nonfunction of the graft, and graft loss.[3-5] Several ex vivo machine perfusion techniques have been explored to reduce preservation injury and to assess graft functionality and viability before transplantation.

Oxygenated hypothermic machine perfusion (oxHMP) has been explored more extensively in recent years. Although animal studies show conflicting results regarding the benefits of this approach,[6-9] a recent clinical trial found that oxHMP results in increased 1-y graft survival compared with non-oxHMP.[10] HMP is easy to perform and can be used during transportation, but graft assessment is limited. Preclinical studies found that normothermic ex vivo kidney perfusion (NEVKP) is superior to non-oxHMP and static cold storage.[11-13] Clinical studies investigating the safety and benefits of NEVKP are few, but early results are promising.[14,15] NEVKP reduces cold ischemic injury and provides opportunities for graft assessment, but it is challenging to perform, and at the moment, no portable device is available for transportation. In the current study, we assessed whether a combination of oxHMP followed by a short NEVKP period could combine the advantages of both preservation techniques. Kidney injury and graft function were assessed in vivo for 8 d of follow-up.

MATERIALS AND METHODS

Study Design and Animals

Twelve-week-old male Yorkshire pigs (~30 kg) were used. Before starting the study, approval was obtained by the Animal Care Committee of the University Health Network Research Institute, Ontario, Canada.

Pigs were assigned to 1 of the 3 groups (Figure 1). All pig kidneys were exposed to 30 min of warm ischemia (WI) followed by 16 or 19 h of perfusion. Three ex vivo perfusion techniques were compared. Kidneys either underwent 16-h NEVKP or were preserved by 16-h oxHMP. The third group was treated with 16-h oxHMP followed by 3-h NEVKP (oxHMP + NEVKP group). After contralateral nephrectomy, grafts were autotransplanted and animals were followed up for 8 d. During the postoperative period, blood was collected daily and fluids and antibiotics were administered twice daily as previously reported.[16] At the end of the follow-up period, animals were euthanized under anesthesia. All animals received humane care, and all procedures were performed in accordance with the "Principles of Laboratory Animal Care" and the "Guide for the Care of Laboratory Animals." Notably, the experiments of all 3 groups were performed within the same period and study design. The results from the oxHMP group have previously been reported in a different study.[17]

Kidney Retrieval and Autotransplantation

Our protocols for anesthesia and kidney retrieval and transplantation have been previously described.[16,17] Following anesthesia induction with subcutaneous midazolam and ketamine, animals were intubated and general anesthesia was maintained by the administration of inhaled isoflurane. A central venous catheter was placed into the internal jugular vein for blood collection and administration of fluids and medications. The next step is sterile disinfection and coverage of the surgical field, followed by a midline incision and dissection of the right kidney and its vessels. To simulate DCD conditions, the renal artery and vein were clamped. Grafts were retrieved after 30 min of WI, the renal artery and vein were cannulated (in all groups), and the grafts were flushed with 300 mL histidine–tryptophan–ketoglutarate (HTK) containing 10 000 IU/L of heparin (4 °C). After the flush, the grafts were connected to the perfusion device for the preservation period. The abdomen was checked for bleeding, then closed with a running suture. After recovery from anesthesia, animals were returned to their pen. Toward the end of the preservation time, animals were brought back to the operating room and reanesthetized. Following reintubation, anesthesia was maintained by inhaled isoflurane and continuous intravenous propofol. The midline laparotomy was reopened, and the left kidney was removed. The stored kidney was then flushed with 300 mL heparinized HTK (4 °C), and the renal anastomoses were sewed (renal vein end-to-side to vena cava, renal artery end-to-side to the aorta, and donor ureter side-to-side to recipient ureter). The abdomen was again checked for bleeding, closed, and the animals were recovered and returned to their pen.

NEVKP

Our NEVKP system has been described in previous publications.[17,18] The perfusion system consists of an

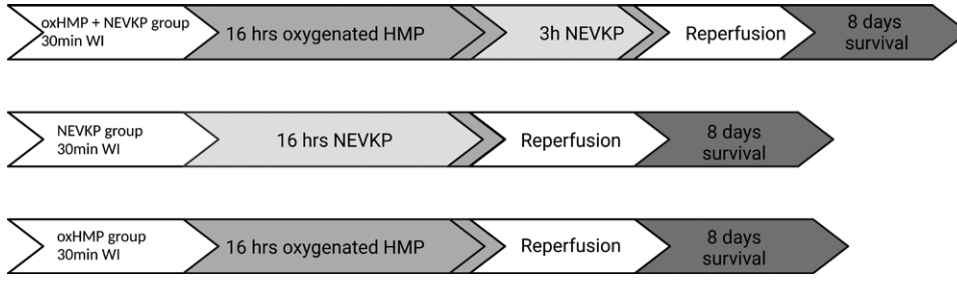

FIGURE 1. Study groups. Pigs were assigned to 1 of the 3 groups (n = 5 in the oxHMP and oxHMP + NEVKP group; n = 3 in the NEVKP group). After 30 min of warm ischemia, kidneys were retrieved and preserved with either 16-h oxHMP (oxHMP group) or 16-h NEVKP (NEVKP group). The third group was treated with 16-h oxHMP followed by 3-h NEVKP (oxHMP + NEVKP group). Following perfusion and after contralateral nephrectomy, grafts were autotransplanted and animals were followed up for 8 d. NEVKP, normothermic ex vivo kidney perfusion; oxHMP, oxygenated hypothermic machine perfusion; WI, warm ischemia.

S3 heart-lung machine and neonatal cardiopulmonary bypass equipment including a centrifugal pump, an oxygenator, a venous reservoir, an arterial bubble filter, and polyvinyl chloride tubing (Sorin Group Inc., Markham, Canada). A heat exchanger was built into the system to regulate temperature. Perfusate temperature, arterial and venous pressure, and arterial flow were continuously recorded. The perfusate solution is made of Ringer's lactate (200 mL), STEEN solution (XVIVO Perfusion AB, Goteborg, Sweden; 150 mL), washed leukocyte-filtered pig erythrocytes (125 mL), double reverse osmosis water (27 mL), sodium bicarbonate (8.4%; 8 mL), calcium gluconate (10%; 1.8 mL), and heparin (2000 IU).[12,18] pO_2 levels were maintained at 650 mmHg during the entire preservation time via continuous oxygen/carbon dioxide gas (95%/5%; 2 L/min) administration. A vasodilator and a mix of amino acids, glucose, and insulin were administered continuously during perfusion.[19] Arterial pressure was initially set at 75 mmHg and maintained at 65 to 70 mmHg by adjusting the rate of the centrifugal pump. Ringer's lactate was continuously infused at a rate of 10 mL/h to account for the loss of circulating volume because of urine production.

oxHMP

The NEVKP system described earlier was modified to perform hypothermic machine perfusion.[17] Instead of a heat exchanger, a heater–cooler device was attached to maintain the temperature at 3 to 6 °C throughout the perfusion. Belzer's Machine Perfusion Solution (1.5 L) was used to prime the system. The chamber in which the organ was placed was surrounded by ice. To maintain pO_2 levels at around 650 mmHg, oxygen (75 mL/min) was added to the system continuously using a membrane oxygenator for the whole preservation time. Similar to the LifePort 1.0 system, arterial pressure was kept at 30 mmHg and the vein was not cannulated. The venous perfusate gathered around the kidney, which was submerged in a machine perfusion solution. The fluid was returned to the reservoir via a roller pump.

For the oxHMP + NEVKP group, at the end of the oxHMP perfusion, the kidney was flushed with 300 mL of HTK (4 °C) and then the organ was placed on ice. The perfusion system was reprimed as presented previously for NEVKP perfusion and then the kidney was connected to the system. This second cold ischemic time was kept at a minimum and was in all cases <15 min.

Perfusate samples were collected hourly and stored at –80 °C for further investigation. During NEVKP, urine samples were also collected regularly. At the end of perfusion, the kidney was removed from the device, reflushed with 300 mL heparinized HTK (4 °C), and stored on ice until transplantation.

Sample Collection and Analysis

Blood gas analyses of the subject were taken before kidney retrieval, before and after transplantation, and every day postoperatively. A point-of-care comprehensive metabolic blood chemistry analyzer (Piccolo Xpress, Union City, Canada) was used to analyze the samples. A part of each sample was snap frozen for later analysis. Blood gas analyses of the perfusate were performed hourly during graft perfusion.

Using a customized metabolic cage, 24-h urine collection was performed before the experiment and on postoperative days (PODs) 2 to 3. For creatinine clearance, serum samples and 24-h urine collection samples were sent to the Toronto General Hospital Core Laboratory for analysis with the Abbott Architect Chemistry Analyzer using the manufacturer's reagents. For the measurement of urinary neutrophil gelatinase–associated lipocalin (NGAL), a porcine NGAL enzyme-linked immunosorbent assay kit was used according to manufacturer's instructions (Bioporto, Hellerup, Denmark).

Histology Assessment

Wedge biopsies were taken from the renal graft at sacrifice. Samples were placed in 10% neutral buffered formalin and transferred to 70% alcohol after 36 to 48 h. Following paraffin-embedding and sectioning (3 μm), periodic acid-Schiff (PAS)–stained sections were used to score global tubular injury and inflammation on a semiquantitative scale of 0 to 3 (0—no changes, 1—mild, 2—moderate, 3—severe changes) by a renal pathologist blinded to the experimental groups.[12,18] For tubular injury assessment, degree of brush border loss, tubular dilatation, epithelial thinning and vacuolation, and luminal debris/casts were evaluated >20 high-power fields and averaged. Interstitial inflammation was scored in 10 low-power fields and averaged.[20]

Endothelial cells were identified by labeling kidney sections with antibodies to CD31 (#NB100-2284, Novus Biologicals) and erythroblast transformation-specific–related gene (ERG; #ab92513, Abcam). Sections were also stained with antibodies to CD3 (#790-4341, Ventana) to quantify the inflammatory T-cell infiltrate. Slides were scanned using a whole slide scanner for brightfield (Axio Scan.Z1, Carl Zeiss Microscopy GmbH, Jena, Germany), and positively stained cells were quantified using image analysis software (HALOTM Image Analysis Software, Pelkin Elmer, Waltham, MA). For ERG and CD3 determination, results were expressed as a percentage of positive cells, and for CD31 as a percentage of positive area.

Statistical Analysis

R software was used for the statistical analyses (version 4.1.1). Descriptive statistics were calculated (mean ± SD) and tests were conducted to compare variables among the study groups and subgroups. The Shapiro-Wilk normality test was conducted to determine whether the data were normally distributed. An analysis of variance test was used to compare continuous variables with a normal distribution. The Kruskal-Wallis test was used to compare continuous variables with nonnormal distribution. When significance was reached, *t* tests and Wilcoxon tests, respectively, were performed to determine which 2 groups were significantly different. A paired *t* test was used to test the significance of differences in normally distributed continuous parameters over time within the same group. Significance was defined as a P value of <0.05.

RESULTS

Animal Characteristics

Average animal weight was similar among groups (NEVKP: 31.9 ± 2.2 kg, oxHMP: 29.1 ± 1 kg,

oxHMP + NEVKP: 29.5 ± 1.1 kg, $P = 0.13$). In all 3 groups, all animals survived until the end of the study.

Perfusion Parameters

Kidney grafts subjected to oxHMP alone demonstrated improvement in flow rates and a decrease in the intrarenal vascular resistance (IRR) during perfusion ($P = 0.38$, respectively $P = 0.42$). For the group that received both oxHMP + NEVKP, a similar trend to the oxHMP group was observed during cold perfusion. The flow rate at the end of perfusion was higher than that at the baseline (values measured at 5 min after the perfusion was started, $P = 0.05$), whereas the IRR decreased during perfusion ($P = 0.11$; Figure 2). For the NEVKP group, renal blood flow progressively increased during the perfusion ($P = 0.0028$), whereas the IRR significantly decreased ($P = 0.04$). For the group that received end-ischemic NEVKP, a similar trend was observed; flow significantly increased during the 3 h of perfusion ($P = 0.0016$), whereas the IRR significantly decreased ($P = 0.02$; Figure 2).

The lactate concentration in the perfusate increased slightly during oxHMP perfusion (baseline: 0.19 ± 0.1, 15 h: 0.47 ± 0.31 mmol/L, $P = 0.12$); for the group that received oxHMP + NEVKP, a similar trend to the oxHMP group was observed during cold perfusion (baseline: 0.61 ± 0.12, 15 h: 0.65 ± 0.34 mmol/L, $P = 0.77$; Figure 3). Electrolyte concentrations (sodium, potassium, calcium, and chloride) and the acid–base parameters (pH, bicarbonate, base excess) remained within physiologic range during the entire perfusion. Lactate significantly decreased throughout NEVKP perfusion (baseline: 9.5 ± 1 versus 15 h: 1.5 ± 0.12 mmol/L, $P = 0.0047$). For the oxHMP + NEVKP group, lactate also significantly decreased during the 3 h of NEVKP perfusion (baseline: 7.4 ± 1.2 versus 3 h: 2.4 ± 1 mmol/L, $P = 0.00023$; Figure 3). All kidneys produce urine during the perfusion.

Posttransplant Graft Function and Injury

Grafts preserved by continuous and end-ischemic NEVKP showed improved function with lower peak serum creatinine (SCr) and more rapid recovery compared with the oxHMP group (peak SCr NEVKP versus oxHMP versus oxHMP + NEVKP: 5.7 ± 0.9 mg/dL versus 9 ± 5.5 mg/dL versus 3.9 ± 1.4 mg/dL). The differences in daily SCr levels reached significance between NEVKP and oxHMP on POD 7 and 8 ($P = 0.01$ and $P = 0.047$, respectively) and between NEVKP and oxHMP + NEVKP on POD1 ($P = 0.04$). Similarly, there was a significant difference in daily SCr

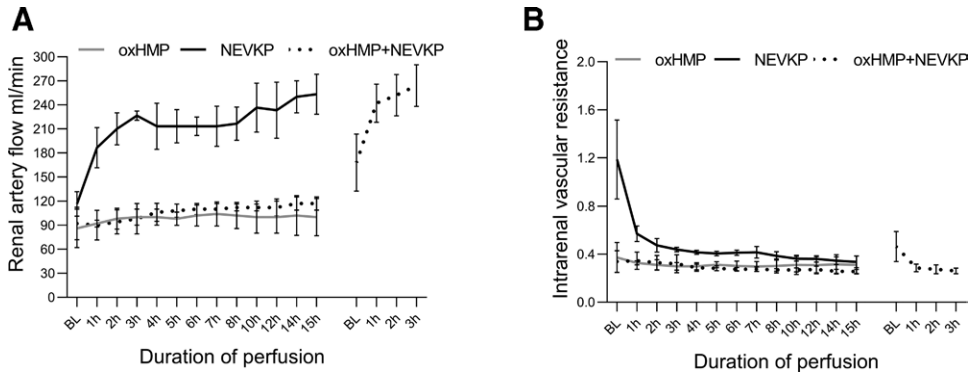

FIGURE 2. Pump parameters. All values are presented as mean ± SD (n = 3–5 in each group). A, Renal arterial flow. B, Intrarenal vascular resistance. In all 3 groups, there was an improvement in the renal arterial flow and a decrease in the IRR (for the oxHMP group, no significance was reached, *t* test, $P = 0.38$ and $P = 0.42$, respectively; for the NEVKP group, renal arterial flow significantly increased, *t* test, $P = 0.0028$, whereas IRR significantly decreased, $P = 0.04$; for the oxHMP + NEVKP group, flow rate increased during the cold perfusion, *t* test, $P = 0.05$, whereas IRR decreased without reaching significance, *t* test, $P = 0.11$; during the end-ischemic NEVKP phase, flow significantly increased during perfusion, *t* test, $P = 0.0016$, whereas IRR significantly decreased, *t* test, $P = 0.02$). BL, baseline; IRR, intrarenal vascular resistance; NKVKP, normothermic ex vivo kidney perfusion; oxHMP, oxygenated hypothermic machine perfusion.

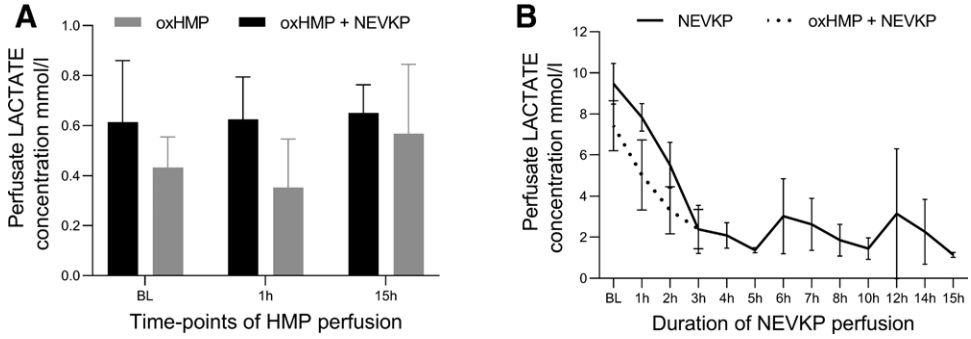

FIGURE 3. Perfusate lactate concentration. A, Perfusate lactate during cold perfusion. Perfusate lactate slightly increased during oxHMP in both the oxHMP group and the oxHMP + NEVKP group (no significance was reached, *t* test, $P = 0.12$, $P = 0.77$, respectively). B, Perfusate lactate during NEVKP perfusion. During continuous and end-ischemic NEVKP, lactate significantly decreased (*t* test, $P = 0.0047$ and $P = 0.00023$, respectively). BL, baseline; NEVKP, normothermic ex vivo kidney perfusion; oxHMP, oxygenated hypothermic machine perfusion.

between oxHMP + NEVKP and oxHMP on PODs 1, 2, and 7 ($P = 0.002$, $P = 0.04$, and $P = 0.016$, respectively; Figure 4). On POD3, creatinine clearance was increased in the NEVKP and oxHMP + NEVKP groups (NEVKP versus oxHMP versus oxHMP + NEVKP: 41 ± 20 mL/min versus 13 ± 13 mL/min versus 33 ± 13 mL/min, $P = 0.072$; Figure 4).

The area under the curve analysis of the SCr from POD1 to POD8 showed no significant difference between the 3 groups (all $P > 0.05$).

Postoperative urine NGAL was measured and normalized to urinary creatinine concentration. Urine NGAL was found to be significantly lowered on POD3 in the oxHMP + NEVKP group compared with the oxHMP group ($P = 0.008$). Urine NGAL was also lowered in the NEVKP group compared with the oxHMP group, although significance was not reached. Serum NGAL measurements were lowest in the group that received continuous NEVKP at 10 h after reperfusion and on PODs 1 and 2. Also, the oxHMP + NEVKP group had lower serum NGAL values than the oxHMP group (Figure 5).

The area under the curve analysis of the postoperative NGAL from 10 h after reperfusion until POD2 showed no significant difference between the 3 groups (all $P > 0.05$).

Renal tissue samples collected on POD8 and assessed with PAS-stained slides showed tubular injury scores to be similar across the 3 groups ($P > 0.05$; Figure 6A). However, inflammation scores were different between groups, highest in the NEVKP group and reaching significance between the NEVKP and the oxHMP + NEVKP groups ($P = 0.03$; Figure 6B).

CD3 staining identified significantly more positive cells in the NEVKP group compared with the oxHMP group ($P = 0.037$) and the oxHMP + NEVKP group ($P > 0.05$; Figure 6C), in keeping with the observation on the inflammatory infiltrate in PAS-stained slides. A representative picture of the image analysis output is shown in **Figure S1** (**SDC**, http://links.lww.com/TP/C826).

Peritubular capillary endothelial cell density measured by CD31 and ERG staining was comparable among the 3 groups (all $P > 0.05$; **Figure S2, SDC,** http://links.lww.com/TP/C826). A representative picture of the image analysis output is also shown in **Figure S2** (**SDC**, http://links.lww.com/TP/C826).

DISCUSSION

In the present study, we investigated whether a combination of oxygenated HMP followed by a short NEVKP

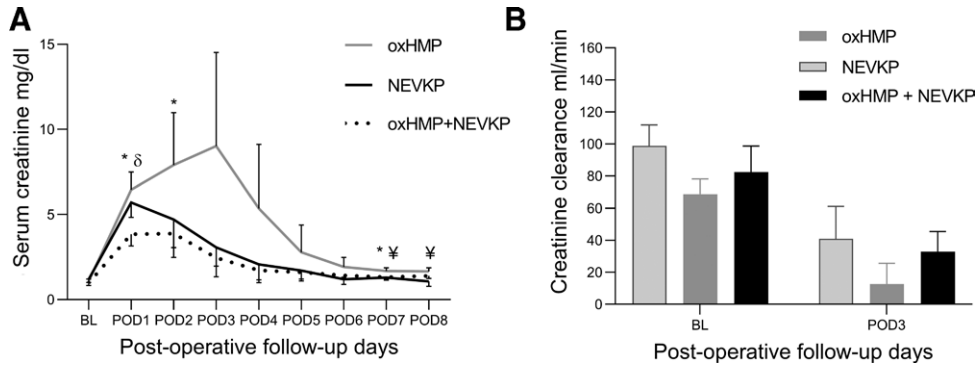

FIGURE 4. Posttransplant graft function. All values are presented as mean ± SD (n = 3–5 in each group). A, Serum creatinine of transplanted animals during the 8 d follow-up. Overall, there was a significantly lower serum creatinine in the NEVKP group and the oxHMP + NEVKP group compared with the oxHMP group. ¥Significance difference between NEVKP and oxHMP on PODs 7 and 8, *t* test, $P = 0.01$ and, respectively, $P = 0.047$; δsignificant difference between NEVKP and oxHMP + NEVKP on POD1, *t* test, $P = 0.04$; *significant difference between oxHMP + NEVKP and oxHMP on PODs 1, 2, and 7, *t* test, $P = 0.002$, $P = 0.04$, $P = 0.016$, respectively). B, Creatinine clearance at baseline and POD3. Creatinine clearance on POD3 was higher in the NEVKP group and the oxHMP + NEVKP groups vs the oxHMP group (Wilcoxon test, $P = 0.072$). BL, baseline; NEVKP, normothermic ex vivo kidney perfusion; oxHMP, oxygenated hypothermic machine perfusion; POD, postoperative day.

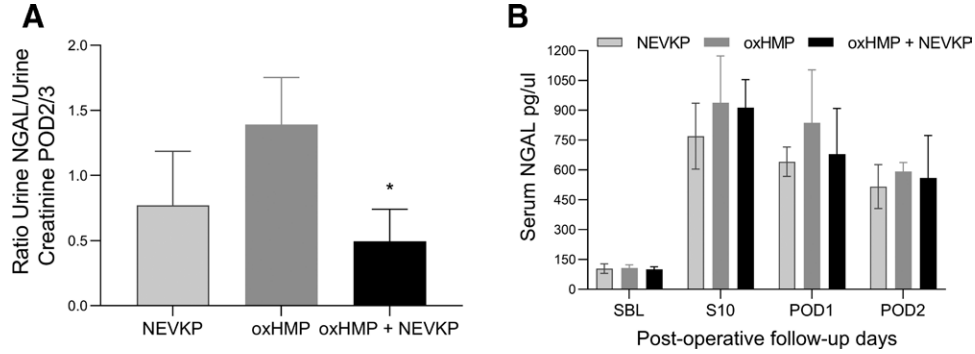

FIGURE 5. Urinary and serum NGAL. A, Urinary NGAL. NGAL measured in the urine from 24-h collection from POD2 to POD3 was normalized to urinary creatinine concentration. *Significance between the oxHMP + NEVKP group and the oxHMP group (Wilcoxon test, $P = 0.008$). B, Serum NGAL. NGAL was measured in serum SBL, at 10 h after reperfusion (S10), on POD1, and on POD2. NEVKP, normothermic ex vivo kidney perfusion; NGAL, neutrophil gelatinase-associated lipocalin; oxHMP, oxygenated hypothermic machine perfusion; POD, postoperative day; SBL, sample taken at baseline.

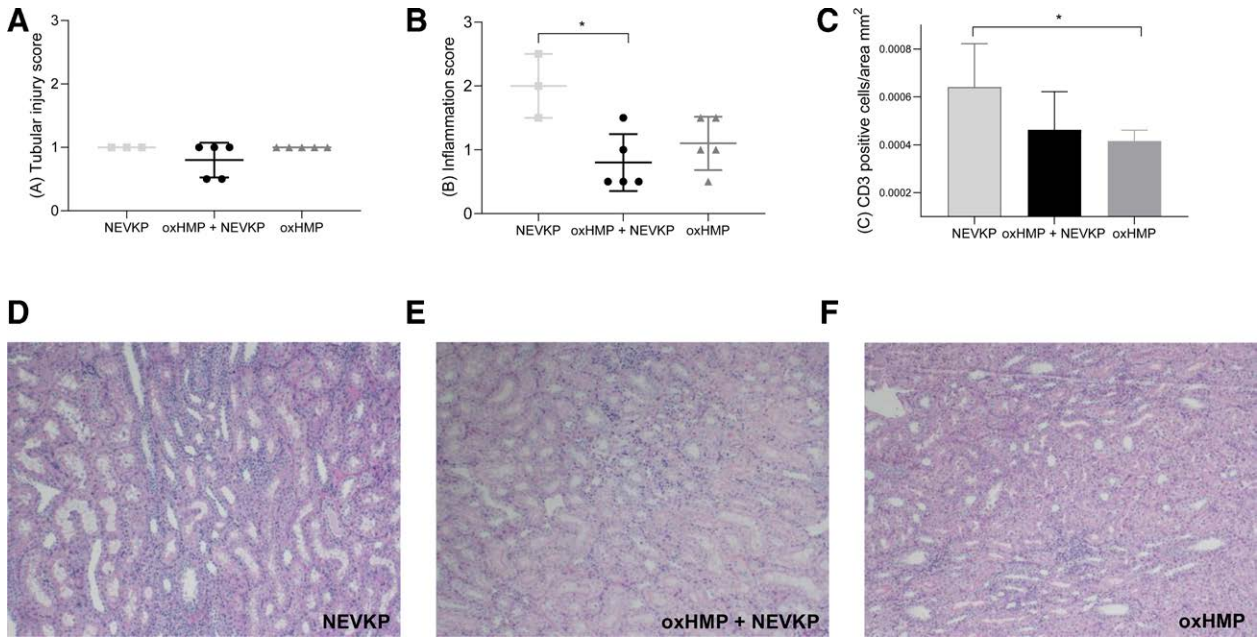

FIGURE 6. Histopathologic changes among the 3 experimental groups. A, Graphical representation of tubular injury score comparing NEVKP, oxHMP + NEVKP, and oxHMP groups, respectively, showing similar injury in all 3 groups. B, Graphical representation of inflammation score comparing NEVKP, oxHMP + NEVKP, and oxHMP groups, respectively, showing greater inflammation in the NEVKP group (*significance between the NEVKP and the oxHMP + NEVKP group, the Wilcoxon test, $P = 0.037$). C, Graphical representation of the CD3 labeling PAS-stained representative images showing the degree of tubular injury as well as inflammation in the NEVKP (D), oxHMP + NEVKP (E), and oxHMP (F) groups (all at 10×). NEVKP, normothermic ex vivo kidney perfusion; oxHMP, oxygenated hypothermic machine perfusion; PAS, periodic acid-Schiff.

period could combine the advantages of both preservation techniques. We found that grafts preserved with oxHMP followed by end-ischemic NEVKP have similar graft function compared with grafts preserved with NEVKP from procurement until transplantation. Both groups had initial superior graft function compared with oxHMP alone. Grafts preserved with continuous and end-ischemic NEVKP demonstrated lower peak SCr, improved creatinine clearance on POD3, and lower urine NGAL as markers of kidney injury. Histology on POD8 showed no differences in tubular injury between groups, in keeping with kidney function as measured by SCr values, which had nearly normalized in all groups by day 8. Animals receiving continuous NEVKP had higher inflammation scores on POD8 compared with the other 2 groups. This aligns with previous studies from our group, in which we found enhanced inflammatory transcriptomic signal grafts preserved with NEVKP. Increased inflammation could be related to repair mechanisms during NEVKP because inflammation is essential in tissue repair and regeneration.[21]

Darius et al[6] found that oxHMP is superior to non-oxHMP, followed by end-ischemic NEVKP. In this study, grafts were exposed to 30 min of WI and either 22 h of oxHMP or 20 h of non-oxHMP followed by 2 h of warm perfusion. Animals were followed for 13 d after perfusion and transplantation. The area under the curve analysis of SCr was lower for the oxHMP group compared with the HMP + NEVKP group. Daily SCr (values normalized to pig body weight) were also lower in the oxygenated HMP group. This is not in line with our findings; however, the lack of oxygenation during the cold preservation time and the slightly shorter NEVKP perfusion time could contribute to the different findings.

A recent European clinical trial found less graft loss and fewer acute rejection episodes in DCD grafts preserved using continuously oxygenated versus nonoxygenated cold perfusion.[10] The delayed graft function rate and the estimated glomerular filtration rate at 1 y were similar between the 2 groups. The addition of a few hours of NEVKP end-ischemic could further enhance the benefits of oxHMP for graft preservation and offer a platform for graft treatment.

NEVKP maintains active metabolism, allowing for graft assessment and possible therapeutic intervention. However, no portable perfusion device is currently available for NEVKP, and warm perfusion remains demanding to plan and perform. On the contrary, oxHMP is easier and can be performed during transportation. Graft assessment and treatment are limited because of the low temperature. The present findings have multiple implications from a clinical point of view. The combination of oxHMP followed by end-ischemic NEVKP is currently achievable in a clinical setting. Grafts can be transported with oxHMP to the transplant center and then connected to an NEVKP device for assessment and treatment. One of the major issues in accepting kidney grafts for transplantation is the reliance on donor data. There are a number of nonmodifiable donor characteristics (eg, age, diabetes history, sex) that cannot be influenced by any preservation method. However, there are also modifiable factors, such as the degree of WI injury, which could potentially be influenced. The donation pool could be significantly increased if all organs could be connected to an NEVKP system for objective assessment. Moreover, graft therapy is possible due to the active metabolism during NEVKP. Implementing NEVKP only at transplant centers is logistically easier to achieve than having a portable NEVKP

device. Hamelink et al[22] recently reviewed the literature on NEVKP and the protocols that are currently being used in different centers. The authors point out that the great diversity among perfusion methods and the readouts of viability biomarkers are currently an issue; more standardized protocols are necessary to facilitate the translation of NEVKP in the clinic.

One of the challenges of normothermic machine perfusion is the necessity to use a perfusate based on red blood cells. Venema et al[23] investigated a colloid-based serum-like solution as a perfusate alternative during NEVKP. The authors could demonstrate that this colloid solution could support metabolism during perfusion; however, kidneys perfused with a blood-based perfusate showed superior function.

Controlled oxygenated rewarming for the preservation of kidney grafts has also been intensively investigated in the past years. Ogurlu et al[24] found that slow rewarming leads to improved tubular function and energy levels during NEVKP and has the potential to reduce rewarming injury. The effects of slow rewarming on kidney function after transplantation and its benefits compared with NEVKP and oxHMP remain to be determined in future studies.

Our study has several strengths. Considering the anatomical and physiological similarities between pigs and humans, our large animal model is translatable in a clinical setting. Moreover, kidney injury and function could be assessed in this in vivo model. Because graft injury is a complex process not limited to solely the ischemic period but which continues after reperfusion, the transplantation survival model is best suited to understand the ramification of these processes.

We recognize that our study also has several limitations. The small number of animals per group may have been underpowered to detect certain differences and some of our observations may not be reproducible in larger studies. Also, due to the autotransplantation model, the potential immunological effects of the ex vivo preservation methods are not apparent. The short follow-up period does not allow us to assess long-term graft function. Moreover, the lack of prepreservation graft injury, moderate kidney injury (30 min WI), and use of healthy animals as recipients might have resulted in an earlier recovery of graft function. The optimal duration of NEVKP for graft assessment and repair remains unclear; therefore, it is debatable whether 3 h of normothermic perfusion could be sufficient.

In summary, this study found that in a pig kidney DCD transplant model, continuous and end-ischemic NEVKP after oxHMP resulted in improved initial graft function compared with oxHMP alone. Grafts preserved with continuous and end-ischemic NEVKP performed similarly. Faster recovery with NEVKP might lead to superior and higher long-term graft survival. In a clinical setting, combining oxHMP with end-ischemic NEVKP could be a practical alternative to combining the advantages of both preservation techniques. Adding a short period of NEVKP at the end of the perfusion opens new avenues for potential graft assessment and treatment.

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