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Published in:
American Journal of Transplantation

DOI:
10.1016/j.ajt.2024.01.002

Publication date:
2024

American Journal of Transplantation xxx (xxxx) xxx

Contents lists available at ScienceDirect

American Journal of Transplantation

journal homepage: www.amjtransplant.org

Brief Communication

Red blood cells as oxygen carrier during normothermic machine perfusion of kidney grafts: Friend or foe?

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ARTICLE INFO

Keywords:
red blood cells
kidney transplantation/nephrology
organ perfusion and preservation
oxygen carrier
normothermic machine perfusion

ABSTRACT

Renal ex vivo normothermic machine perfusion (NMP) is under development as an assessment tool for high-risk kidney grafts and as a means of achieving more physiologically accurate organ preservation. On-going hemolysis has been reported during NMP, as this technique relies on red blood cells for oxygen delivery. In this study, we confirm the occurrence of progressive hemolysis during 6-hour kidney NMP. NMP-associated erythrostasis in the glomeruli and in peri-glomerular vascular networks points to an interaction between the red blood cells and the graft. Continuous hemolysis resulted in prooxidative changes in the perfusate, which could be quenched by addition of fresh frozen plasma. In a cell-based system, this hemolysis induced redox stress and exhibited toxic effects at high concentrations. These findings highlight the need for a more refined oxygen carrier in the context of renal NMP.

Abbreviations: DBD, donation after brain death; fHb, free hemoglobin; GSTA1, glutathione s-transferase alpha 1; HBOC-301, hemoglobin-based (artificial) oxygen carrier-301; HMOX1, heme-oxygenase 1; NMP, normothermic machine perfusion; NFE2L2, nuclear factor erythroid 2-related factor 2; NQO1, NAD(P)H quinone dehydrogenase 1; RBC, red blood cell.

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https://doi.org/10.1016/j.ajt.2024.01.002
Received 21 August 2023; Received in revised form 16 December 2023; Accepted 1 January 2024
Available online xxxx

1. Introduction

Ex vivo normothermic machine perfusion (NMP) is a new development that aims at more physiologically accurate preservation, conducting pretransplant functional assessment, and reconditioning perceived high-risk kidney grafts.[1-3] This technique, designed to restore physiological metabolic activity at or near 37 °C, is heavily dependent on the incorporation of an oxygen carrier.

In order to ensure clinical compatibility, most NMP protocols opt for stored red blood cells (RBCs) as the preferred oxygen carrier.[4-7] Although RBCs have been successfully applied in liver NMP procedures,[8,9] significant hemolysis and microvascular obstructions caused by RBC aggregates have been reported in the context of renal NMP.[10,11] Given the potential profound implication of these findings in the realm of renal NMP, we sought to validate these observations and assess their potential impact on renal grafts.

2. Materials and methods

2.1. Donor kidneys

The joint Medical Ethical Committee for Human Research in Leiden, Den Haag, and Delft granted approval (number B19.019) for the hypothermic pulsatile machine perfusion (NMP) protocol, which involved donor kidneys deemed unsuitable for transplantation (n = 15). These discarded kidney grafts underwent 6 hours of perfusion under normothermic conditions. Permission was obtained from the relatives of the donors for the utilization of the kidneys for research purposes.

Prior to NMP, the grafts were either stored using static cold storage (University of Wisconsin CS solution, Bridge to Life, DC, USA) or by NMP (Kidney Assist Transport, XVIVO, Göteborg, Sweden; LifePort Kidney Transporter, Organ Recovery Systems, Itasca, IL, USA) with the modified (University of Wisconsin MPS, Bridge to Life) preservation solution. Organs were flushed using Ringer's solution prior to the start of NMP.

Clinical post-reperfusion cortical biopsies (n = 11) were collected after 45 minutes of reperfusion of static-cold-stored kidneys.[12] Six grafts were from donation after cardiac death and 5 from donation after brain death (DBD) donors. Three DBD and 3 donation after circulatory death grafts developed delayed graft function; the other 5 had immediate function. All grafts showed a full functional recovery.

2.2. Normothermic machine perfusion set-up and perfusate composition

NMP was performed using the Kidney Assist device (XVIVO). Clinical-grade saline-adenine-glucose-mannitol-preserved leukocyte-reduced RBCs (O-negative, stored for a maximum of 7 days) were obtained from a certified blood bank (Sanquin, Amsterdam, The Netherlands). Blood donors consented to the use of their product for research. Prior to use, the RBCs were subjected to a washing process (CellSaver equipment, Sorin, Italy) with 2L NaCl 0.9% according to the manufacturer's protocol. Perfusate composition and machine settings used during perfusion are provided in Supplementary Table 1.

The device was primed with perfusate at 37 °C, the perfusion pressure controlled at 75 mmHg, and the perfusate was oxygenated with oxygen and carbon dioxide gas (95% O_2/5% CO_2) at a rate of 0.5 L/min.

Glucose and pH were monitored and titrated to maintain a glucose concentration within the range of 5 to 7 mmol/L and a pH between 7.35 and 7.45. Any urine produced was recirculated to the perfusate every half hour.

Sequential perfusate samples and cortical punch biopsies (Ø 4 mm) were collected prior to and at 1-, 3-, and 6 hours of NMP. Blood gas and biochemical analyses were performed using the RAPIDPoint 500 Blood Gas Analyzer (Siemens, Munich, Germany). Free hemoglobin (fHb) in the perfusate supernatant and urine (1300 g, 15 minutes) was measured at the certified Leiden University Medical Centers clinical chemistry laboratory using spectrophotometry. Injury markers, including kidney injury molecule-1 and human neutrophil gelatinase-associated lipocalin, were measured using quantitative sandwich enzyme-linked immunoassays (respectively DY1750B and DY1757, Bio-Techne, Minneapolis, MN, USA) according to the manufacturer's instructions.

In order to evaluate a possible pro-oxidant activity of the RBC alternative hemoglobin-based (artificial) oxygen carrier (HBOC)-301 (HbO2 Therapeutics LCC, Souderton, PA, USA), HBOC was prepared similar to baseline perfusate (final perfusate HBOC concentration: 4.1 mmol/L) and evaluated.

2.3. Impact of the NMP system on the RBCs

The potential impact of the NMP circuit on hemolysis was assessed by circulating perfusion fluid in the circuit in the absence of a kidney graft. To simulate conditions to standard NMP, a section of the tubing was partially clamped, thereby creating a resistance and flow rate similar to that during a typical renal NMP procedure.

2.4. Immunohistochemistry

Tissue biopsies were formalin-fixed and sectioned into 4-μm slices. Erythrocyte membranes and fibrin deposition were respectively visualized through antibodies against glycophorin CD235a (M0819, Agilent Technologies, CA, USA) and fibrin (reference: 0541; Immunotech, Marseille, France) following citrate heat-retrieval. Visualization was achieved using EnVision Polymer and DAB (Agilent Technologies). Positive (aortic aneurysm for glycophorin; placenta for fibrin) and negative controls were included. Images were captured using digital microscopy (Philips IntelliSite Pathology Solution Ultra-Fast Scanner, Eindhoven, The Netherlands).

The degree of glycophorin staining per glomerulus was estimated using QuPath.[13] Glomeruli were manually annotated, and the area featuring positive glycophorin was measured using a

thresholder in the Pixel Classification function. This allowed for the quantification of glycophorin positive area per glomerulus.

2.5. Transmission electron microscopy

For morphologic analysis, small tissue sections (1 mm^3) were fixed in 1.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, UK) in 0.1 M cacodylate buffer at room temperature for 1 hour. After a brief storage time (in 0.5% paraformaldehyde in 0.1M cacodylate buffer at 4 °C), the samples were postfixed in 1% OsO4/1.5% potassium hexanocyano-ferrate (III)/0.1M cacodylate buffer on ice. Afterward, the samples were dehydrated with a series of ethanol followed by infiltration with mixtures of propylene oxide (Electron Microscopic Sciences) and EPON (LX112, Ladd Research, VT, USA). The tissues were orientated, mounted in a mold with EPON, and polymerized at 60 °C for 48 hours.

Ultrathin sections (90 nm) were collected on one-hole grids and stained with a 7% uranyl acetate solution and Reynold's lead citrate solution. Sections were imaged with a twin electron microscope (Tecnai T12 Twin, Thermofisher, Eindhoven, The Netherlands) with a Gatan OneView Camera (AMETEK, Berwyn, PA, USA), and photographs were stitched together, resulting in a large image.[14]

2.6. Lipid peroxidation assay

Possible changes in perfusate pro-oxidant activity were assessed by monitoring induction of albumin-bound linoleic acid (3.22 mM; L1376, Merck, Darmstadt, Germany) oxidation using the Oroboros Oxygraph-2 K (Oroboros Instruments, Innsbruck, Austria).[15,16]

2.7. Cell culture

Proximal porcine tubular epithelial cells (LLC-PK-1, LGC Standards S.a.r.l., Molsheim, France) were cultured in 12-well plates using DMEM (Dulbecco's Modified Eagle's Medium, 1443001, ThermoFisher, Waltham, MA, USA) supplemented with 10% deactivated fetal bovine serum, glucose (25 mM), GlutaMax (4 mM; 35050061, Merck, Darmstadt, Germany), and penicillin/streptomycin (5 µL/mL, P0781, Merck). Cells were grown to confluence and then maintained for 24 hours. Next, cells were exposed for 24 hours to perfusate samples (10% in culture medium, collected at the end of NMP) with varying fHb concentrations, HBOC containing baseline perfusate, or appropriate controls.

2.8. Real-time polymerase chain reaction analysis in tissue and cells

Tissue biopsies (immediately stored at -80 °C) were powdered in liquid nitrogen and homogenized using the QIAshredder kit (Qiagen, Hilden, Germany). Cultured cells were dissociated using trypsin ethylenediaminetetraacetate and homogenized using the QIAshredder kit.

RNA isolation was performed using the RNEasy miniKit (Qiagen) according to the manufacturer's instructions. The messenger RNA quality and quantity were evaluated with the NanoDrop (ThermoFisher) bioanalyzer. When adequate messenger RNA quality was obtained (260/280 ration), complementary DNA was synthesized with the High-Capacity RNA-to-cDNA kit (Thermo Fisher). Real-time polymerase chain reaction was performed on QuantiStudio 5 Real-Time PCR System (Thermo Fisher).

Taqman gene expression assays (Thermo Fisher) were used to quantify the expression of the following target genes of the nuclear factor erythroid 2-related factor 2 (NRF-2) signaling pathway, which responds to oxidative stress: nuclear factor erythroid 2-related factor 2 (*NFE2L2*) [Hs (human) 00232352_m1, Ss (porcine) 06886078_m1], heme-oxygenase-1 (*HMOX1*) [Hs01110250_m1, Ss03378516_u1], glutathione s-transferase alpha 1 (*GSTA1*) [Hs00747232_mH, Ss04323031_m1], and NAD(P)H quinone dehydrogenase 1 (*NQO1*) [Hs00168547_m1, Ss04246169_m1]. Expression levels are given as ratios to glyceraldehyde 3-phosphate dehydrogenase [4308313, Life Technologies; Ss03374854_g1], and the change in expression calculated using delta-delta Ct method (mean ± standard deviation).

2.9. Statistics

Data are expressed as mean ± standard deviation for normally distributed data or as median (interquartile range [IQR]) for not normally distributed data. Differences between groups were analyzed using the independent sample *t* test and analysis of variance. Differences between the start and end of perfusion were estimated using the paired sample *t* test. Two-sided *P* values of $<.05$ were considered significant. Statistical analyses were performed using SPSS 25.0 statistical software.

3. Results

This study is based on 15 kidney grafts that were deemed unsuitable for transplantation and that underwent 6 hours of NMP. Donor and retrieval characteristics are displayed in Supplementary Table 2. Median age of the RBCs was 6 days (range, 2-7 days). Preperfusion washing of the RBCs resulted in a median fHb start concentration of 5.0 (IQR, 4.0-6.0) µmol/L. Total Hb concentration remained consistent throughout the perfusion, measuring 5.2 ± 1.6 mmol/L at the beginning and 4.6 ± 1.0 mmol/L at the end of the perfusion ($P = .205$). Details of the perfusion characteristics are provided in Supplementary Fig. S1.

The median fHb levels rose to 58 (IQR, 43-79) µmol/L at the end of perfusion (after 6 hours of NMP [$P < .001$]) (Fig. 1). Following 6 hours of NMP, only a minimal degree of hemolysis (final fHb concentration of 6 µmol/L) was observed in absence of a kidney in the circuit ($n = 2$), implying involvement of the kidney graft in the induction of hemolysis observed during renal NMP. No associations were found between the arterial flow or renal resistance index and the fHb concentrations ($r = 0.512$, $P = .074$).

A significant correlation was found between the fHb concentration in perfusate and urine ($r = 0.870$, $P < .0001$) (Supplementary Fig. S2). Urine fHb concentrations ranged from 0.04 to 12.8 µmol/L (approximately 20-fold lower than in the perfusate, Supplementary Fig. S2).

End of NMP fHb levels did not correlate with the renal injury markers kidney injury molecule-1 ($r = -0.361$, $P = .186$) and

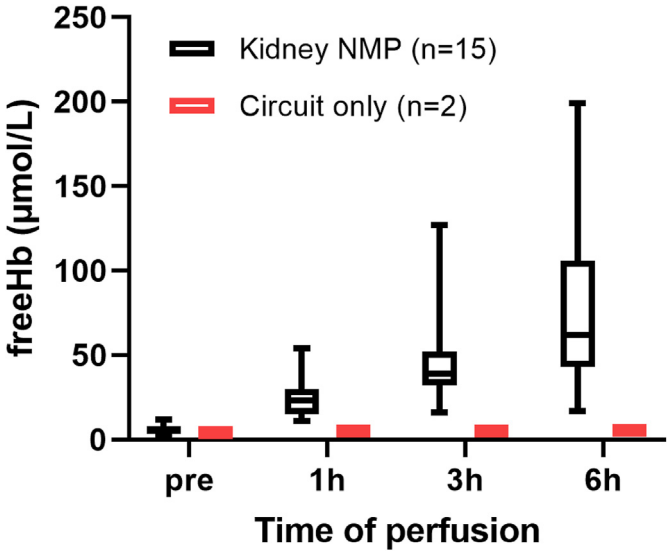

Figure 1. Free hemoglobin increases during renal normothermic machine perfusion (black box plots, kidney normothermic machine perfusion [NMP], n = 15). Minimal hemolysis in the absence of a kidney graft in the perfusion circuit (red box plots, circuit only, n = 2). Box-Whisker plots depict the median, interquartile range, and extremes.

neutrophil gelatinase-associated lipocalin (r = 0.182, $P = .571$), Supplementary Fig. S3.

A possible interaction between RBCs and the graft was confirmed through glycophorin A staining and transmission electron microscopy (TEM). Imaging showed progressive, localized accumulation of RBC aggregates within the peritubular capillaries and glomeruli (Fig. 2A, B) during the course of perfusion. Thus, this phenomenon was distinct to the NMP procedure as it was not observed in the reperfusion phase of clinical transplantation or prior to NMP (Fig. 2C and Supplementary Fig. S4). No correlation was found between the extent of glycophorin staining and the degree of hemolysis (r = -0.130; $P = .646$) or the arterial flow (r = -0.272; $P = .369$) (Supplementary Fig. S5).

Baseline erythrostasis was influenced by the type of storage: static-cold-stored grafts (n = 6) showed significantly more erythrostasis at the start of NMP ($P < .001$) than grafts preserved on HMP. After 6 hours of NMP, significantly more erythrostasis was present in DBD grafts ($P = .018$) (Supplementary Fig. S5D). RBC aggregates were negative for fibrin (Supplementary Fig. S6), and TEM excluded rouleaux formation (Fig. 2B) as the cause of the intervascular aggregates. A cold flush after NMP did not ameliorate the erythrostasis (Supplementary Fig. S4E).

The cytotoxicity of free Hb and heme primarily arises from their pro-oxidant activities. Possible changes in perfusate pro-oxidant activity were assessed by monitoring linoleic acid auto-oxidation. Baseline perfusate solution (ie, without RBCs) did not induce lipid peroxidation (data not shown). In contrast, the introduction of fHb-containing perfusate collected during NMP resulted in an immediate induction of auto-oxidation. This pro-oxidant activity positively correlated with perfusate fHb levels (Fig. 3) and was antagonized by the inclusion of fresh frozen plasma (perfusate/plasma ratio 1:1) in the medium. Evaluation of HBOC-301, a potential off-the-shelf alternative for RBCs, showed

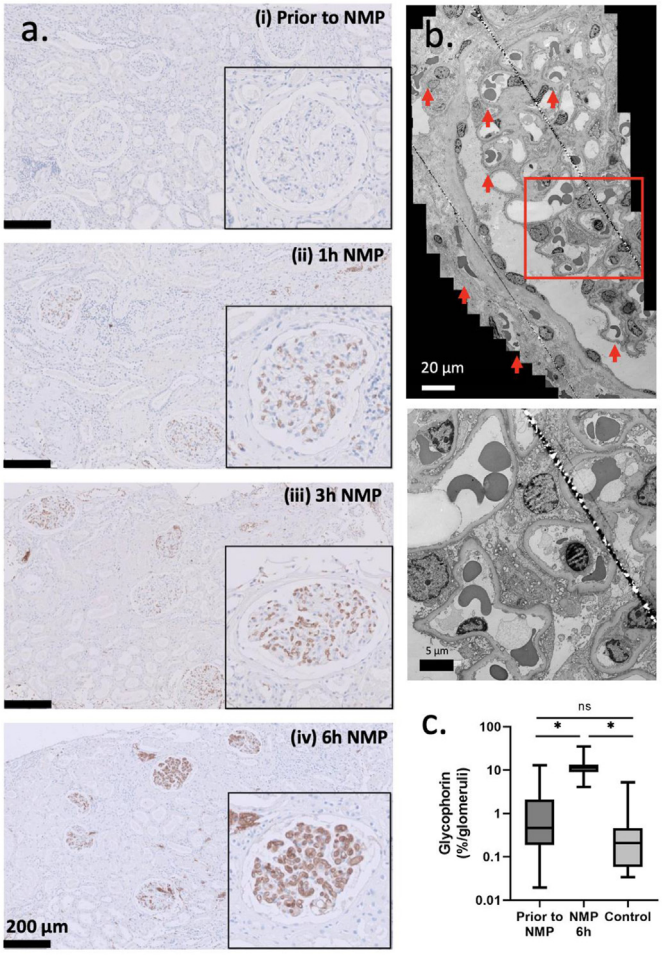

Figure 2. Progressive glomerular and peritubular erythrostasis during normothermic machine perfusion (NMP). (A) Absent erythrostasis (glycophorin staining, CD235a) in biopsies collected prior to NMP (i) and progressive erythrostasis during NMP (ii) at 1 hour, (iii) at 3 hours, and (iv) at 6 hours. (B) Transmission electron microscopy imaging of a glomerulus after 6 hours of NMP. Intact red blood cells, with no evident rouleaux formation (red arrows). The bar is 20 µm. (C) Quantification of the degree of erythrostasis (glycophorin area per glomerulus). Box-Whisker plots illustrate the degree of erythrostasis following 6 hours of renal NMP. Controls represent biopsies collected 30 minutes after clinical transplantation of grafts procured using static cold storage. Absent erythrostasis shows a minimal glycophorin signal, similar to prior to NMP ($P = .205$). Box-Whisker plots depict median, interquartile range, and extremes. *$P < .0001$.

profoundly reduced pro-oxidant activities compared with fHb (Supplementary Fig. S7A).

To test whether the accruing fHb levels observed lead to cellular (redox) stress, we monitored the expression of constituents of the NRF-2 redox-response pathway upon exposure of the LCC-PK-1 proximal tubule epithelial cell line to "native" (RBC-free) perfusate as well as NMP perfusate with varying fHb concentrations. Although native perfusate did not activate the pathway, exposure to perfusate obtained during NMP led to progressive, partial activation of the NRF-2 pathway (for intermediate fHb concentrations) or cytotoxicity (for perfusate with the highest fHb concentrations (approximately $> 10^6$ µmol/L).

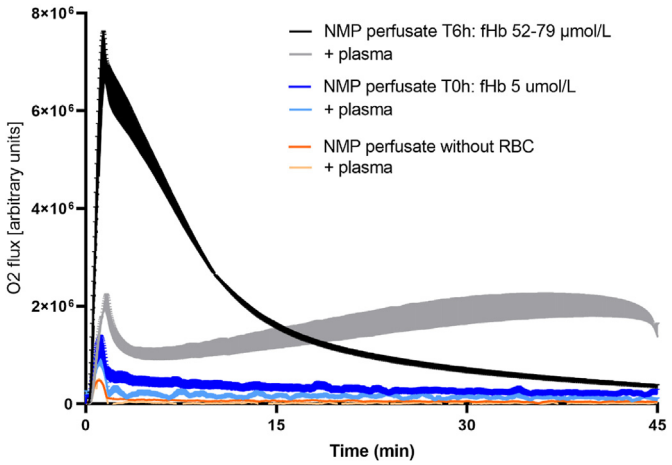

Figure 3. Induction of linoleic acid oxidation is measured using oxygen flux upon exposure to perfusate. Absent lipid oxidation upon exposure to native perfusate (orange curve). Concentration-dependent induction of lipid peroxidation by free hemoglobin (fHb)-containing normothermic machine perfusion (NMP) samples (blue curves [5 µmol/L], black curves [>50 µmol/L]). Inclusion of plasma quenches lipid peroxidation induced by fHb-containing NMP samples (light blue curves [5 µmol/L], gray curves [>50 µmol/L]). RBC, red blood cell.

Perfusates with intermediate fHb concentrations exhibited a dose-dependent upregulation of *HMOX1* and *NQO1* genes. Independent of the fHb concentrations, perfusate suppressed the expression of *NFE2L2* and *GSTA1* (Fig. 4). Exposure to HBOC containing perfusate showed a modest induction of *HMOX1* and *NQO1* expression (Supplementary Fig. S7B).

Data obtained from the perfused kidneys mirrored the results observed in the cell-based assay, showing a (median) 2.5-fold increase ($P = .005$) in tissue *HMOX1* expression after 6 hours NMP. Expression of *NQO1, GSTA1,* and *NFE2L2* remained stable (Fig. 5).

4. Discussion

Although liver NMP has found its way into clinical practice, renal NMP presents greater challenges. In light of this, DiRito et al[10] and Hosgood et al[11] reported hemolysis and microvascular obstructions attributed to the utilization of stored RBCs. Considering the critical need for inclusion of RBCs as oxygen carriers during NMP, we sought to explore the challenges associated with the use of banked RBCs in the context of renal NMP.

The results of this study concur with earlier observations of progressive hemolysis during renal NMP involving RBCs.[10,11] In fact, median fHb concentrations exceeded thresholds considered acceptable within the clinical context (eg, 31 µmol/L for patients on extracorporeal membrane oxygenation).[17] Although it is acknowledged that mechanical pumps can contribute to hemolysis, the results of NMP perfusions conducted without a kidney in the circuit showed minimal hemolysis over a 6-hour perfusion, pointing at a primary, causative role for the kidney graft.

Histologic examination validated the presence of progressive erythrostasis within the glomerular and postglomerular venules during and at the end of NMP, as was also reported by DiRito et al[10] and Hosgood Et [11] However, minimal obstructions were observed prior to NMP. This contrast may be related to differences in handling of the organ prior to NMP (flush/hypothermic perfusion).

Furthermore, using a diagnostic-grade antibody, we could not confirm a claimed role for fibrin deposition in the formation of intravascular erythrocyte clusters.[18] Moreover, TEM excluded rouleaux formation, gross erythrocyte deformation, or a clear interaction between the vessel wall and the erythrocyte as underlying triggers. Note that erythrocyte clusters formed during NMP could not be removed by flushing the organ. To test whether the observed erythrostasis is NMP-specific, a series of kidney biopsies collected 45 minutes after clinical transplantation were stained. The absence of erythrostasis within the (peri)glomerular networks of these transplanted kidneys suggests that the observed erythrostasis is NMP-specific.

The kidney is particularly vulnerable to the consequences of hemolysis.[19] Both heme and fHb function as Fenton reagents, displaying strong pro-oxidative properties.[20] Evidence suggests that in hemolytic diseases, after an initial stimulus that activates the endothelium and/or causes RBC lysis, heme and fHb act as a second "hit," leading to intolerable endothelial stress and resulting in tissue and organ injury.[21] Under physiologic circumstances and mild hemolysis, any fHb or heme is effectively

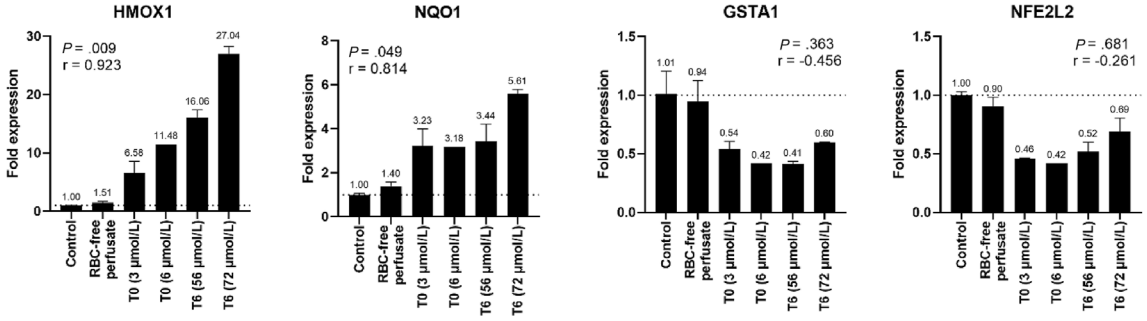

Figure 4. Change in relative messenger RNA expression of signature genes of the nuclear factor erythroid 2–related factor 2 (NRF-2) redox-response signaling pathway upon exposure to perfusate with varying free hemoglobin levels in renal epithelial LLC-PK1 cells. Dose-dependent upregulation of heme-oxygenase-1 (HMOX1) ($P = .009$) and NAD(P)H quinone dehydrogenase 1 (NQO1) ($P = .049$) expression, but absent expression of glutathione S-transferase alpha 1 (GSTA) and nuclear factor erythroid-derived 2-like 2 (NFE2L2). Box-Whisker plots depict the median, interquartile range, and extremes. RBC, red blood cell.

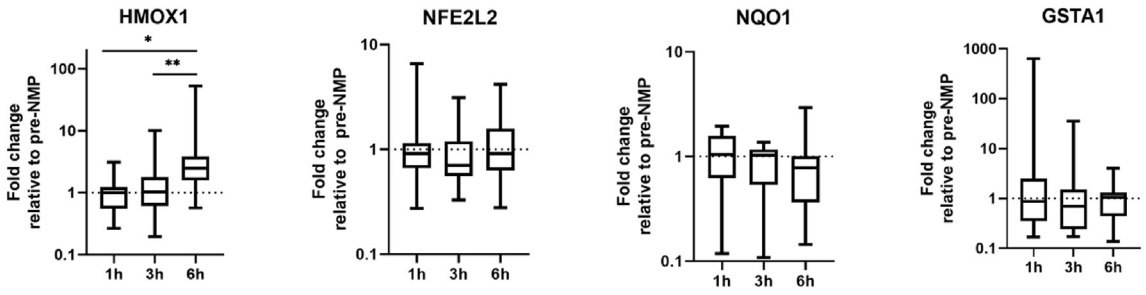

Figure 5. Change in graft relative messenger RNA expression of signature genes of the NRF-2 redox-response signaling pathway during normothermic machine perfusion (NMP), normalized to baseline expression from similar kidney grafts prior to NMP. Upregulation of heme-oxygenase-1 (HMOX1), but stable glutathione S-transferase alpha 1 (GSTA1), nuclear factor erythroid 2-related factor 2 (NFE2L2) and NAD(P)H quinone dehydrogenase 1 (NQO1) expression. Box plots indicate median, IQR and extremes. Box-Whisker plots depict the median, interquartile range, and extremes. * $P < .01$, ** $P < .001$.

scavenged and inactivated by the plasma proteins haptoglobin and hemopexin, and these complexes are then cleared by the liver.[22,23] Failure of the scavenging mechanism during excess hemolysis exceeding the body's fHb and heme binding capacity can result in severe renal damage.[19,24]

A possible, but speculative, explanation for the hemolysis observed during renal NMP is that graft metabolic competence is not achieved under the current perfusion conditions. Consequently, the metabolic interaction between the RBCs and their host might be inadequate to re-establish or sustain erythrocyte metabolic competence.[25] Absence of progressive hemolysis during liver NMP[26] suggests that the perfused liver might be able to sustain an environment that is required for erythrocyte metabolic health. Introduction of a hemodialysis filtering system could also be a potential solution to prevent lactate accumulation (and thus preventing lactate dehydrogenase product inhibition in the RBCs and kidney) and filter out excess fHb and other waste products.

We used induction of linoleic oxidation to monitor possible changes in perfusate pro-oxidant activity.[16] Although no oxidation was observed when linoleic acid was exposed to native perfusate, immediate and spontaneous induction of lipid peroxidation occurred upon exposure to perfusate samples collected after 6 hours NMP to the albumin-linoleic acid mixture. This, indicates profound changes in the pro-oxidant state of the perfusate during NMP, similar to those observed for clinical conditions with severe intravascular hemolysis.[16] In order to cope with intravascular hemolysis, plasma is equipped with several classes of antioxidants that prevent reactive oxygen species formation or neutralize reactive oxygen species formation.[27] Indeed, fHb-induced pro-oxidant activity was effectively counteracted by the inclusion of fresh frozen plasma, suggesting that incorporation of plasma, or the hemoglobin/heme scavengers haptoglobin and hemopexin in NMP protocols could potentially offer beneficial effects.

We applied a cell-based system to test whether fHb results in cellular stress. The agile NRF-2 redox-response network is the cell's fast acting response pathway to redox stress,[28] that includes genes such as HMOX-1.[29] Activation of this pathway dominates the graft's early response to reperfusion following clinical transplantation.[30] Exposure of the LCC-PK-1 porcine proximal tubules cell line to perfusate collected during renal NMP but when exposed to native perfusate resulted in a partial activation of the NRF-2 pathway and a dose-dependent induction of heme-oxygenase expression, the principal heme-detoxifying enzyme for low and intermediate fHb concentrations. Clear cytotoxic effects were observed in this in vitro system for those perfusate samples with the highest fHb content. A similar pattern of partial induction of the NRF-2 pathway, ie, of heme-oxygenase expression, was observed in perfused kidneys, showing that perfusate fHb triggers a cellular response in the perfused graft.

The observed hemolysis during renal NMP and its potential cytotoxic effects constitute a challenge for the clinical implementation of renal NMP and call for further optimization of NMP conditions or use of safer alternatives to RBCs. An acellular perfusate would be beneficial considering its ease of storage and handling, availability for use, and minimal antigenicity. Although acellular HBOC has been proposed as a possible alternative, our data indicate some pro-oxidative activity, and the safety of HBOC for renal NMP remains to be established.[31]

This study is limited by several factors, including the unknown clinical impact of NMP because these kidneys were not transplanted. Because the kidneys were declined for transplantation due to multiple reasons, the group is rather heterogeneous. It is unknown whether the conclusions from perfusions with partial, inferior quality kidney grafts translate to perfusions of clinical-grade quality kidney grafts.

In conclusion, this study found that persistent hemolysis during renal NMP results in a toxic environment that is potentially harmful to the graft. Inclusion of fresh frozen plasma rather than albumin solution may provide a much-needed first line of antioxidant defense. Moreover, current protocols may require further optimization in order to better sustain a renal metabolism that is not only able to support the metabolic recovery of stored RBCs, but also addresses the metabolic burden associated with the use of RBCs (eg, lactate clearance). In this context, the potential of improved RBC preservation techniques or RBC rejuvenation prior to NMP deserves attention.[32]

Acknowledgments

The authors thank the donors and their families for their permission and trust in the use of discarded donor kidneys for research purposes, and the Dutch organ donation coordinators for their logistic help. The authors also thank Jason Doppenberg

(LUMC) and Lianne Stevens (LUMC, TNO) for their assistance with perfusion procedures. Connie van Rhijn provides assistance with immunohistochemistry procedures. Carolina Jost and Roman Koning (LUMC) obtained transmission electron microscopy images.

Funding

This study was supported by the Dutch Kidney Foundation (PROPER Study; BHF1P02) and the PROPER team (Leiden University Medical Center, University Medical Center Groningen and Erasmus MC, The Netherlands).

Data availability

The data that support the findings of this study are available on request from the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2024.01.002.

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