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Brief hypothermic oxygenated perfusion provides cardioprotection in a pig model of donation after circulatory death

Manuel Egle [a,b,c], Adrian Segiser[a,b], Alexia Clavier[a,b,c], Georgia Beer[a,b,c], Anja Helmer[a,b,c], Rahel Ottersberg[a,b,c], Selianne Graf[a,b,c], Maria Arnold [a,b], Fabio Zulauf[a], Deborah Lagger[a], Maris Bartkevics[a], Alexander Kadner[a], Daja Krummenacher[d], Peter Vermathen[d], Matthias Siepe [a] and Sarah Longnus [a,b,*]

[a]Department of Cardiac Surgery, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland
[b]Department for BioMedical Research, University of Bern, Bern, Switzerland
[c]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland
[d]Magnetic Resonance Methodology Group, Institute of Diagnostic and Interventional Neuroradiology, University of Bern, Bern, Switzerland

* Corresponding author. CVD Program, DBMR, University of Bern, Murtenstrasse 28, CH-3008 Bern, Switzerland. Tel: +41-31-684-0412; e-mail: sarah.henninglongnus@insel.ch (S. Longnus).

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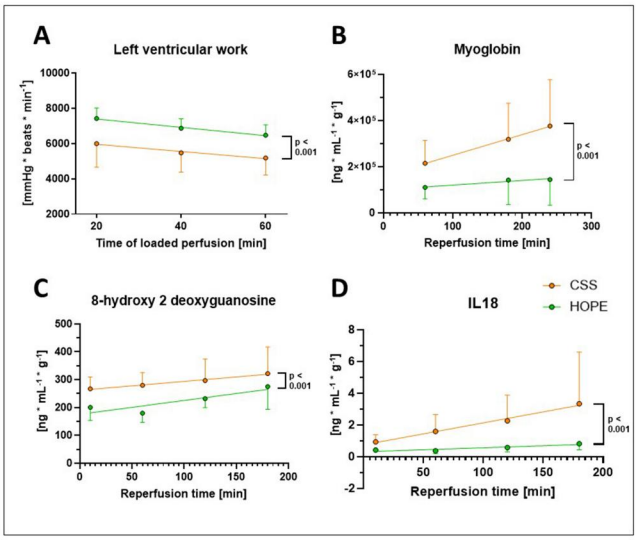
Summary

Brief hypothermic oxygenated perfusion (HOPE), applied between cardiac graft procurement and normothermic machine perfusion, provides cardioprotection in a pig model of donation after circulatory death (DCD). HOPE improved left ventricular function and decreased myocardial cell death, potentially via reduced ischemia- and reperfusion- induced oxidative stress, succinate load, and inflammatory response.



Left ventricular work as a key indicator of functional recovery during loaded perfusion (A). Quantification of the release of the cell death marker myoglobin (B), the marker of oxidative stress 8-hydroxy-2'-deoxyguanosine (C), and the pro-inflammatory cytokine interleukin (IL)-18 (D) during unloaded normothermic machine perfusion. n = 5 per group

Abstract

OBJECTIVES: Donation after circulatory death provides excellent patient outcomes in heart transplantation; however, warm ischaemic graft damage remains a concern. We have reported that a brief period of hypothermic oxygenated perfusion prior to normothermic reperfusion improves graft recovery in a rat model. Here, we investigated the cardioprotective benefits and mechanisms of this approach compared to the current clinical standard in a large animal model.

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METHODS: Circulatory death was induced in anaesthetized male Schweizer Edelschwein pigs (55 kg). Hearts underwent 20 min of warm, *in-situ* ischaemia, followed by a cold coronary flush and explantation. After 15 min backtable preparation, hearts underwent either 15 min cold static storage (control) or 30 min hypothermic oxygenated perfusion. All hearts were perfused *ex vivo* under normothermic conditions; 3 h in an unloaded mode, followed by 1 h with left ventricular loading to assess cardiac recovery.

RESULTS: Compared to control conditions ($n = 5$), hypothermic oxygenated perfusion ($n = 5$) increased recovery of left ventricular function (cardiac output and maximum relaxation rate, $P < 0.001$ for both) and decreased cell death marker release (heart-type fatty acid binding protein, $P = 0.009$ and myoglobin, $P < 0.001$). In parallel, hypothermic oxygenated perfusion reduced the release of succinate and the oxidative stress marker 8-hydroxy-2'-deoxyguanosine.

CONCLUSIONS: A brief period of hypothermic oxygenated perfusion, applied as a reperfusion therapy between graft procurement and normothermic machine perfusion, provides cardioprotection in a porcine model of donation after circulatory death. Hypothermic oxygenated perfusion is a promising, easily applicable, cardioprotective reperfusion strategy; this study provides key evidence to support clinical translation.

Keywords: Heart failure • Heart transplantation • Donation after circulatory death • *Ex vivo/Ex-situ* heart perfusion • Normothermic machine perfusion • Hypothermic oxygenated perfusion

ABBREVIATIONS

CSS	Cold static storage
DCD	Donation after circulatory death
DPP	Direct procurement and perfusion
HOPE	Hypothermic oxygenated perfusion
IL	Interleukin
NMP	Normothermic machine perfusion

INTRODUCTION

Heart transplantation remains the therapeutic gold standard for patients with advanced heart failure [1]. For several years, there has been a discrepancy between the numbers of patients listed for a heart transplant and the organs available for transplantation [2, 3]. Considerable morbidity and mortality on the waiting list led to the reintroduction of utilizing hearts obtained with donation after circulatory death (DCD). This has been successfully implemented by several centres with excellent clinical results [4–7], leading to the further establishment in many centres. Despite excellent results, DCD organ transplantation is associated with global warm ischaemia prior to procurement, potentially leading to severe cardiac graft injury. This can result in reduced post-transplant organ function or discard of the organ [8, 9]. Thus, it is important to develop protocols that minimize ischaemia–reperfusion-associated graft injury to optimize post-transplantation cardiac function [10].

For DCD heart procurement, there are 2 established clinical approaches. One approach, normothermic regional perfusion, involves excluding cerebral circulation and reperfusing hearts in the donors with extracorporeal membrane oxygenation, restoring them to a beating state for assessment. The second approach involves the application of cold preservation solution, followed by direct procurement and reperfusion (DPP) with normothermic machine perfusion (NMP). Clinical outcomes are comparable between the 2 strategies [11]. However, both approaches involve restoring oxygenated perfusion to post-ischaemic hearts under near-normothermic conditions, which may induce significant reperfusion injury, highlighting the need for improved, clinically applicable strategies.

Hypothermic oxygenated perfusion (HOPE) is recognized to alleviate ischaemia–reperfusion injury and improve function in DCD kidney and liver grafts [12, 13]. In DCD pig hearts, replacing graft storage with NMP by HOPE provides comparable outcomes [14]. Moreover, cardiac DCD graft storage with HOPE has only recently been validated as a promising strategy, based on the encouraging short-term outcomes observed in case reports of the first 3 clinical cases [15]. It is important to note that with this HOPE-based approach, hearts are not beating, thereby precluding functional graft assessment. Our group was the first to describe the combined approach, with a brief period of HOPE applied between heart procurement and NMP in a rat model of DCD, demonstrating significantly improved ventricular function and restoration of adenosine triphosphate levels, with reduced myocardial cell death and oxidative stress [16, 17]. Furthermore, we revealed that the beneficial effects of HOPE are dependent on succinate oxidation and nitric oxide production during HOPE and that HOPE reduces reperfusion-related tissue calcium overload [16, 17]. We therefore sought to confirm the superiority of this HOPE-based approach over the currently used DPP protocol in the pig model as the decisive next step in the translation towards clinical application.

MATERIALS AND METHODS

Ethics statement

All experiments were conducted in accordance with the European Convention for the Protection of Animals and approved by the Swiss animal welfare authorities and the Cantonal Veterinary Office, Bern, Switzerland (approval number BE68/2019). All surgical procedures were performed under deep anaesthesia with supervision by a veterinary anaesthetist. All possible measures were taken to minimize the suffering of the animals.

Study design

Two parallel study arms were used to investigate the effects of a brief period of HOPE, compared to the current clinical standard of cold static storage, prior to NMP in a porcine model of DCD with DPP.

Donation after circulatory death protocol

Male pigs (breed: Schweizer Edelschwein) were used. Briefly, systemic heparinization was followed by the cessation of ventilation

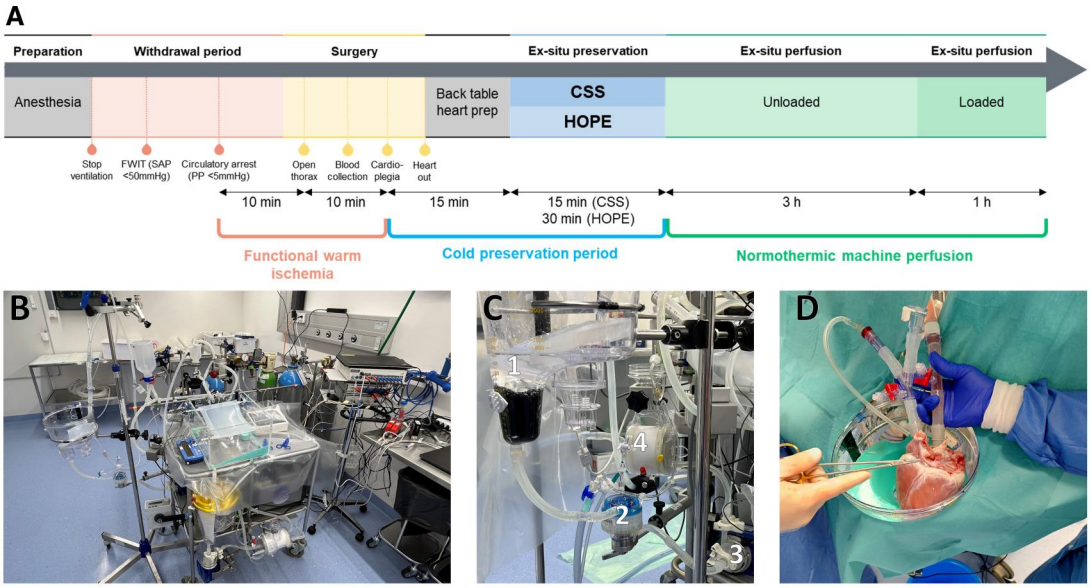

Figure 1: Study design and HOPE perfusion setup. (**A**) Study design with 2 parallel arms. All hearts underwent 20 min of functional warm ischaemia, followed by either 30 min of cold static storage (CSS group), or 15 min cold static storage and 30 min of HOPE (HOPE group). All hearts underwent a total of 4 h of normothermic machine perfusion; 3 h in unloaded mode, followed by 1 h with ventricular loading. (**B**) Custom-made perfusion system for normothermic oxygenated perfusion with HOPE circuit on the left side. (**C**) HOPE perfusion circuit with: 1: reservoir and heart chamber; 2: bubble trap; 3: centrifugal pump; 4: oxygenator. (**D**) Heart preparation during HOPE. CSS: cold static storage; fWIT: functional warm ischaemia; HOPE: hypothermic oxygenated perfusion; PP: pulse pressure; SAP: systolic arterial pressure.

under deep anaesthesia with neuromuscular blockade. After ~10 min of functional warm ischaemia, defined as a drop in systolic blood pressure below 50 mmHg, the chest was opened, and after exactly 20 min of functional warm ischaemia, hearts were procured (Fig. 1). Detailed descriptions of the anaesthesia procedure, DCD protocol and surgical procedure have been reported [18].

Cold preservation period

During cold storage, all hearts were prepared for NMP by cannulating the aorta and left atrium with custom-made cannulae and closing the pulmonary veins and inferior vena cava while leaving the superior vena cava and pulmonary artery open. In cold static storage (CSS) hearts, all these steps were performed on the back table immediately after procurement, and after exactly 30 min of cold static storage, NMP was initiated. For HOPE hearts, in order to start HOPE rapidly, only cannulation of the aorta and closure of the pulmonary veins were performed on the back table, and after exactly 15 min of cold static storage, HOPE was initiated, during which the remaining heart preparation was completed (Fig. 1). An independent perfusion circuit was used for the HOPE (Fig. 1). The circuit included a Puralev® I100SU centrifugal pump (Levitronix; Zurich, Switzerland), an Inspire® VBT 8 bubble trap and a Sorin Inspire® 7F reservoir (both LivaNova; Mirandola, Italy; Fig. 1). A custom-made silicone holder was integrated into the perfusate reservoir/heart chamber as an interface to securely hold the heart. Oxygenation and cooling were achieved using a Capiox® FX 15 oxygenator (Terumo; Tokyo, Japan).

HOPE perfusate consisted of 700 ml of St Thomas No.II cardioplegia, supplemented with 1.5 ml erythropoietin 1000 IU/0.5 ml (Eprex; Janssen, Beerse, Belgium), 100 ml glyceryl trinitrate 1 mg/ml (Sintetica; Mendrisio, Switzerland), 100 ml mannitol 0.2 g/ml (BBraun; Melsungen, Germany), 30 ml tirofiban 12.5 mg/250 ml (Curatis; Liestal, Switzerland), 2 ml methylprednisolone 1 g/15.6 ml (Pfizer; Zurich, Switzerland) and 100 ml washed blood. Perfusate was cooled to a target temperature of 11–12°C using a clinical heater–cooler module and oxygenated to a target oxygen partial pressure >500 mmHg before coronary artery perfusion at a pressure of 30 mmHg, which resulted in coronary flows between 0.3 and 0.6 l/min. HOPE was conducted for exactly 30 min in all hearts from the HOPE group. Upon completion of HOPE, cold saline solution was used to de-air the aortic root prior to connecting the heart to the perfusion system for normothermic reperfusion.

Importantly, a total cold preservation period of 30 min was used in CSS hearts to represent current clinical practice, while a total period of 45 min was used in HOPE hearts, which we expect may be the minimum duration to ensure cardioprotection [16, 17].

Normothermic reperfusion

NMP was performed in the same manner for all hearts using a custom-made perfusion system, which enabled both unloaded (Langendorff) perfusion as well as loading of the left ventricle with adjustable pre- and after-loads (Fig. 1). Donor blood was used to perform *ex-situ* heart perfusion in a 1:1 mixture with a perfusion solution, in accordance with the clinically used protocol. Blood collection was carried out using a cell saver device, which differs from typical clinical practice where whole, unwashed donor blood is used. A detailed description of the NMP protocol has been reported [19].

Functional recovery assessment

During perfusion in working mode, left ventricular function (including left ventricular work, cardiac output, developed pressure, relaxation and contraction rates, triple product) was

continuously recorded. A pressure-volume catheter (Millar; Houston, Texas, USA) was placed in the left ventricle via the left atrium. Quantification of cardiac output and coronary flow was performed using clamp-on tubing flow probes (Transonic Systems; Ithaca, New York, USA). All data were recorded continuously using a PowerLab data acquisition system and the LabChart 8 software (ADInstruments; Oxford, UK).

Perfusate sampling

At regular intervals during HOPE and normothermic perfusion, perfusate was monitored using a Cobas b 123 blood gas analyser (Roche; Basel, Switzerland). Samples of recirculating buffer were collected at regular intervals and centrifuged at 4°C and 2000 g for 5 min. Supernatants were stored at −80°C for later analysis.

Statistical analysis

GraphPad Prism software (GraphPad Software; La Jolla, California, USA) was used for statistical analyses of non-repeated measurements and creation of graphs. SPSS Statistics software (IBM SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis of repeated measurements. Unless otherwise stated, data are presented as mean with standard deviation. Box-and-whiskers plots indicate median, 1st and 3rd quartile (box) and 0th and 100th percentile (whiskers). Data with repeated measurements were analysed by mixed-model linear regression. For non-repeated measures, pairwise comparisons were performed using the Mann–Whitney *U*-test. NMR spectroscopy data was analysed as previously reported [19]. *P*-values were adjusted for multiple comparisons (modified, sequential, rejective Bonferroni procedure) [20] and reported as statistically significant if <0.05.

Additional methods

The following methods are described in the supplementary material: oedema quantification, biochemical analysis of perfusate samples and tissue calcium measurements.

RESULTS

Baseline characteristics

A total of 10 pigs were included in the study, 5 pigs per experimental group. No statistically significant difference between groups was observed for baseline characteristics (Table 1).

Table 1: Baseline characteristics reported as median and interquartile range

Groups	CSS ($n=5$)	HOPE ($n=5$)
Body weight (kg)	51 [48–65]	53 [50–52]
Heart weight (g)	265 [246–327]	298 [263–285]
Interval WLST–fWIT (s)	386 [355–400]	309 [274–524]
Interval WLST–CA (s)	500 [455–501]	544 [513–761]
Maximum arterial pressure (mmHg)	100.7 [99.3–105.7]	94.3 [94.0–103.7]
Minimum arterial pressure (mmHg)	58.7 [51.0–59.0]	50.7 [48.7–57.3]
Mean arterial pressure (mmHg)	74.7 [65.0–80.4]	66.7 [66.3–76.7]
Central venous pressure (mmHg)	8.7 [7.1–9.8]	5.7 [5.3–6.3]

No statistically significant difference was observed between groups for any of the variables.

CA: circulatory arrest; CSS: cold static storage; fWIT: functional warm ischaemia start; HOPE: hypothermic oxygenated perfusion; ns: not significant; WLST: withdrawal of life-sustaining therapy. $n=5$ per group.

Post-ischaemic recovery of left ventricular function

HOPE significantly ($P<0.001$) improved left ventricular function during loading compared to CSS, demonstrated by increased left ventricular work, cardiac output, developed pressure, and maximum relaxation rate (Fig. 2A–D). Maximum contraction rate (Figure 2E) and triple product (product of left ventricular work and maximum contraction rate; Fig. 2F) were similar between groups.

Myocardial cell death

HOPE significantly lowered release of the cell death markers heart-type fatty acid binding protein ($P=0.009$, Fig. 3A) and myoglobin ($P<0.001$, Fig. 3B) compared to hearts preserved with CSS.

Oxidative stress and mitochondrial damage

HOPE led to significantly ($P=0.003$) lower release of 8-hydroxy-2'-deoxyguanosine (Fig. 3C), an indicator of oxidative stress. Cytochrome c release, an indicator of mitochondrial damage, was not different between groups (Fig. 3D).

Perfusate biochemistry

Perfusate biochemistry measured by blood gas analysis: analysis of blood gas data for perfusate samples taken during NMP is presented in Supplementary Material, Table S1. Perfusate lactate levels are presented in Supplementary Material, Fig. S3. Please note: lactate profiles should be interpreted with caution as blood used in normothermic perfusion was washed; this will reduce lactate levels, which in turn can affect cardiac lactate metabolism. Furthermore, HOPE application allowed for the washout of lactate, which would be expected to reduce lactate levels in these hearts compared to the CSS group.

Perfusate biochemistry measured by nuclear magnetic resonance spectroscopy: analysis of HOPE perfusate samples taken at 0- and 30-min time points are presented in Supplementary Material, Table S2. During HOPE perfusion, no net increase in succinate perfusate levels, indicating no succinate release, was observed (Supplementary Material, Table S2). For normothermic perfusion samples measured between 0 and 40 min, orthogonal partial least squares discriminant analysis built for experimental group revealed a clear and significant separation between HOPE and CSS groups ($Q2=0.68$, $P<0.003$; Fig. 4A). During this period, succinate perfusate levels were significantly lower in

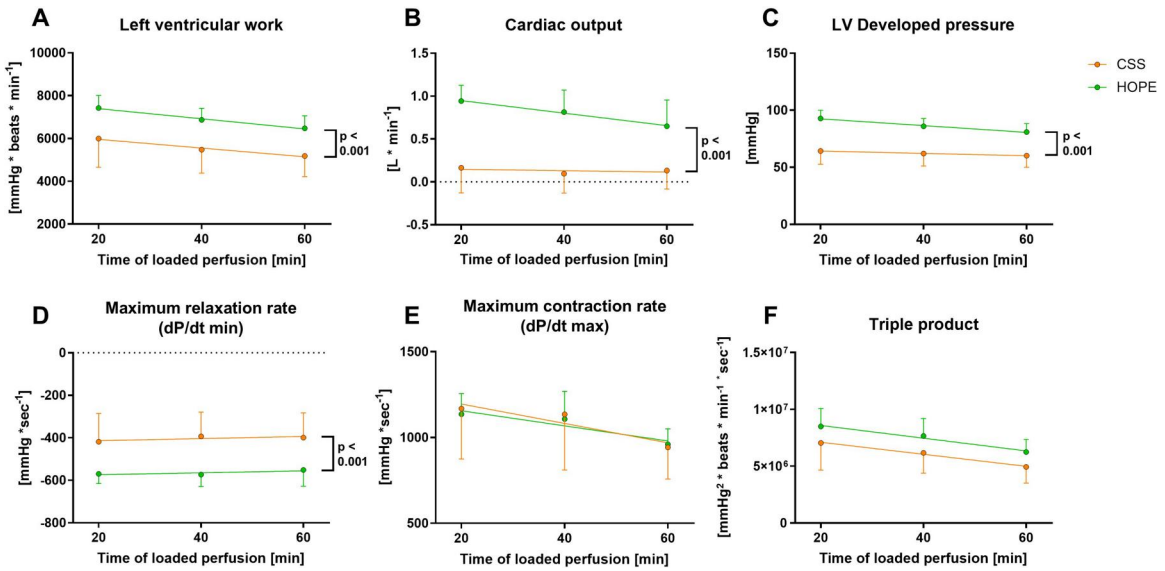

Figure 2: Left ventricular functional recovery during loaded perfusion, expressed as left ventricular work (**A**), cardiac output (**B**), developed pressure (**C**), relaxation rate (**D**), contraction rate (**E**) and triple product (**F**). Left ventricular work is calculated as the product of heart rate and developed pressure. Triple product is calculated as the product of left ventricular work and maximum contraction rate (dP/dt max). CSS: cold static storage; HOPE: hypothermic oxygenated perfusion; LV: left ventricular. $n = 5$ per group.

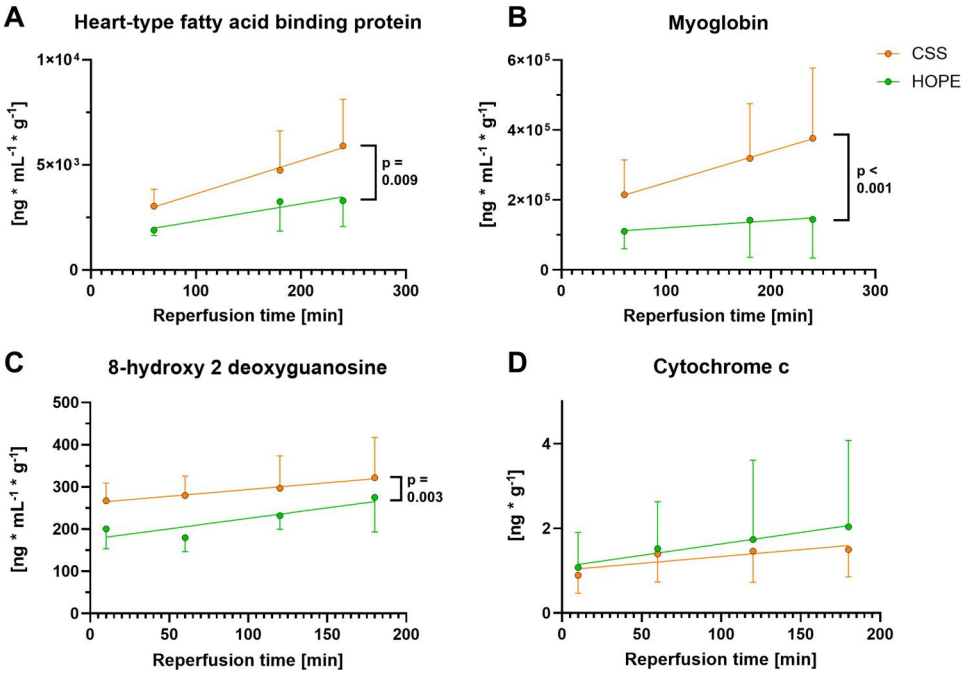

Figure 3: Release of the cell death markers heart-type fatty acid binding protein (H-FABP; **A**) and myoglobin (**B**), the marker of oxidative stress 8-hydroxy-2'-deoxyguanosine (**C**) and a marker for mitochondrial injury cytochrome c (**D**) during unloaded, normothermic machine perfusion. CSS: cold static storage, HOPE: hypothermic oxygenated perfusion. $n = 5$ per group.

HOPE-treated hearts compared to CSS, indicating reduced succinate release compared to CSS ($P = 0.021$; Fig. 4B).

Inflammation

HOPE significantly reduced release of interleukin (IL)-18, IL-10 ($P = 0.006$ for both, Fig. 5A and B) and IL-6 ($P = 0.024$, Supplementary Material, Fig. S1B) compared to CSS hearts. In addition, there was a lower release of interferon gamma (IFN-γ; Fig. 5C) in hearts treated with HOPE compared to CSS that was likely of physiological relevance, but that did not reach statistical significance due to limited statistical power. No differences were observed among groups for tumour necrosis factor alpha, IL-8, IL-12 (Supplementary Material, Fig. S1). Values were below detection level for IL-1α and IL-1β, IL-2, IL-4 and granulocyte-macrophage colony-stimulating factor in both groups (data not shown).

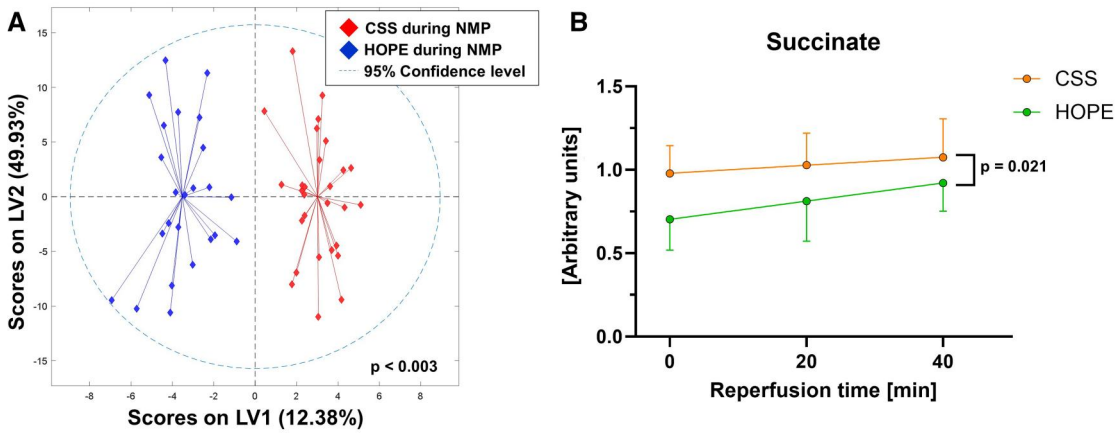

Figure 4: Nuclear magnetic resonance spectroscopy metabolite analysis of perfusate during NMP. (**A**) Orthogonal partial least squares discriminant analysis built on experimental group segregates HOPE and CSS ($Q2 = 0.68$, $P < 0.003$). (**B**) Perfusate succinate levels at early time points. CSS: cold static storage; HOPE: hypothermic oxygenated perfusion; LV: latent variable. n(**A**) = 25 per group, n(**B**) = 5 per group.

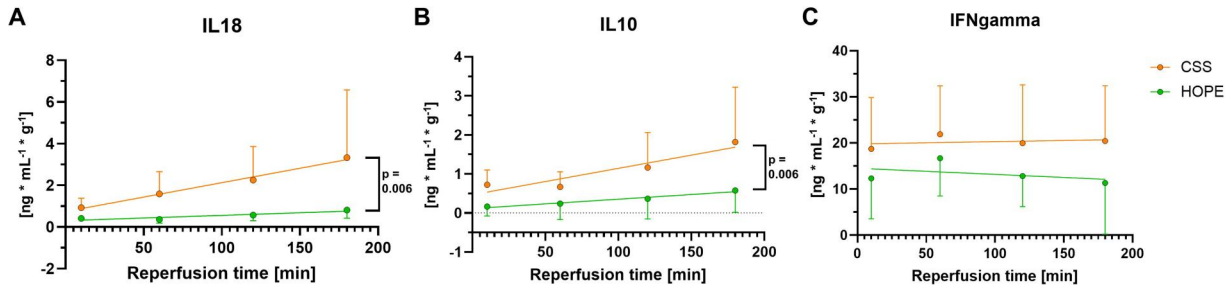

Figure 5: Cytokines released during unloaded, normothermic reperfusion. (**A**) IL-18 belongs to the IL-1 family and represents one of the key pro-inflammatory cytokines in the context of cardiac inflammation. (**B**) IL-10 is an anti-inflammatory cytokine known to be released in the context of cardiac ischaemia and reperfusion and acts to mitigate post-ischaemic necrosis and tissue remodeling. (**C**) The release of IFNγ is known to be triggered by IL-in the context of cardiac inflammation. CSS: cold static storage; HOPE: hypothermic oxygenated perfusion; IFNgamma: interferon gamma; IL: interleukin. $n = 5$ per group.

Coronary flow and oedema formation

There was no difference between groups for either coronary flow during loaded perfusion (Fig. 6A) or for total adenosine administration (Fig. 6B). Although physiologically relevant, the reduction in oedema formation observed with HOPE compared to the CSS group was not statistically significant due to limited statistical power (Fig. 6C).

Tissue calcium content

Tissue calcium content, measured at the end of loaded perfusion, was similar in HOPE and CSS hearts (Supplementary Material, Fig. S2).

DISCUSSION

We demonstrate the cardioprotective effects of a brief period of HOPE, applied between procurement and NMP, as compared with the current clinical standard, CSS, in the direct procurement and perfusion approach using a porcine model of DCD heart transplantation. HOPE significantly improved recovery of left ventricular function and reduced myocardial cell death compared to control hearts. Key mechanistic contributors include diminished oxidative stress and a reduced inflammatory response, which in turn may result from the HOPE-induced lowering of cardiac succinate prior to normothermic reoxygenation. Given its beneficial effects, the application of HOPE may not only improve patient outcomes but may also increase the donor pool by enabling utilization of grafts that otherwise would be considered unsuitable for transplantation.

HOPE increased left ventricular function and decreased the release of myocardial cell death. Taken together, these data support the concept that HOPE improvements in left ventricular function are not simply due to stunning in CSS hearts, but that HOPE provides true cardioprotection.

HOPE significantly reduced oxidative stress compared with CSS, but did not alter the release of cytochrome c, a marker of mitochondrial damage. The HOPE-induced reduction of ischaemia–reperfusion-associated reactive oxygen species is in line with findings from DCD liver experiments; and although there is contradictory information in the literature regarding HOPE-based improvement of mitochondrial function and integrity in different organ systems, our current findings are in agreement with those in rat hearts [16, 17, 21, 22].

Perfusate biochemical analysis with nuclear magnetic resonance spectroscopy demonstrates clear segregation of HOPE and CSS hearts during NMP, when all hearts were exposed to the same conditions, confirming different metabolic profiles between experimental groups. Interestingly, HOPE decreased succinate release during early NMP compared to CSS. Given that no release of succinate was measured during HOPE itself, it is likely that HOPE permitted succinate oxidation at low rates as previously reported [16]. Lower intracellular levels of succinate at the

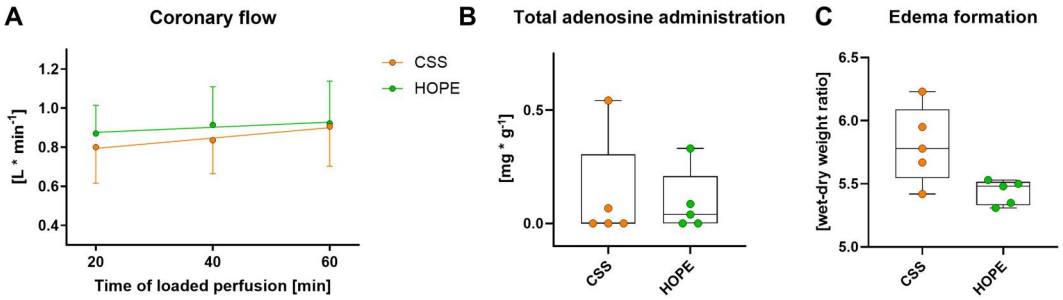

Figure 6. Measurements of vascular function. (**A**) Coronary flow during loaded perfusion. (**B**) Quantification of total adenosine administration during reperfusion. (**C**) Oedema formation measured in biventricular tissue at the end of loaded perfusion. CSS: cold static storage; HOPE: hypothermic oxygenated perfusion. $n = 5$ per group.

onset of NMR would be expected to reduce oxidative stress and inflammatory responses [23], which fit well with our findings.

HOPE altered the inflammatory response during NMP compared to CSS. Notably, HOPE lowered the release of IL-18, which is a pro-inflammatory cytokine belonging to the IL-1 family [24]. Myocardial ischaemia induces the production and caspase-dependent activation of IL-18 [25], and elevated IL-18 levels can lead to reduced ventricular function and apoptosis [26, 27]. Additionally, IL-18 is known to induce the release of IFN-γ [28], in agreement with our observed IFN-γ release in CSS hearts, although the latter did not reach statistical significance due to limited statistical power. Moreover, an elevated release of IL-18 can be perceived as an indication of enhanced activation of the inflammasome [25]. This is of particular interest as targeted inhibition of inflammasome activity in cardiac DCD improves graft quality [29, 30]. HOPE also reduced the release of IL-10, an anti-inflammatory IL with an inhibitory effect on interferon gamma release [31]. Both myocardial ischaemia and reperfusion enhance the release of IL-10, which limits inflammation-based myocardial necrosis and mitigates post-ischaemic remodeling [32–34]. Therefore, the diminished IL-10 release observed in HOPE hearts may result from improved mitigation of ischaemia- and reperfusion injury-related inflammation versus CSS.

Cardiac ischaemia–reperfusion injury causes tissue calcium overload [35]. In this context, HOPE significantly reduced total tissue calcium in the rat heart model [17]. In line with these findings, the significantly improved maximum relaxation rate by HOPE in this study could well be explained by attenuated ischaemia–reperfusion-induced calcium overload. However, in the pig ventricular tissue, no difference in calcium content was observed between HOPE and CSS groups. These differences in findings may be explained by the fact that calcium content determination was performed after 1 h of total reperfusion in rat tissue, but only after 4 h of reperfusion in pigs. It is conceivable that a difference in tissue calcium overload in pig hearts was equilibrated during the additional 3 h of reperfusion, and therefore no longer detectable.

Finally, we observed a physiologically relevant reduction in the development of oedema in HOPE versus CSS hearts, potentially indicating a HOPE-induced decrease in capillary leak; however, this difference was not statistically significant due to limited statistical power. This is in agreement with our findings of reduced IL-18 release with HOPE, as IL-18 can induce apoptosis of human cardiac microvascular endothelial cells [25]. Although the difference in oedema did not reach statistical significance, we believe this finding to be noteworthy as oedema formation is recognized as a time-limiting factor in *ex-situ* cardiac perfusion that is difficult to overcome.

We report above cardioprotective benefits of a brief HOPE prior to normothermic reperfusion in a large animal model of DCD; however, the optimal HOPE protocol remains to be determined. Indeed, several factors are likely critical in the determination of optimal conditions for HOPE, such as duration, temperature, perfusion pressure/flow, oxygen delivery, ATP repletion, succinate depletion and oxidative stress management—factors that are likely highly interdependent. Furthermore, it may be that optimal HOPE conditions also depend on the duration of the preceding ischaemia. As such, next steps towards clinical application should address these issues in priority.

Limitations

Although we provide evidence for HOPE-induced benefits for cardiac graft quality following warm ischaemia, we have not performed experiments aimed at optimizing either application conditions or duration of HOPE in order to obtain the maximal cardioprotective effect. Furthermore, we have used a single functional warm ischaemia duration of 20 min. Although this represents a clinically relevant duration, we cannot draw conclusions about the effect of HOPE over the entire spectrum of possible fWIT durations (~5–30 min). In addition, we have not included histological analyses of the hearts; evaluation of specific markers may help to identify key mechanisms of HOPE-induced cardioprotection. These above-mentioned aspects would be important to investigate in future studies.

CONCLUSIONS

In this study, we have demonstrated for the first time that a brief period of HOPE prior to NMP provides cardioprotection in a porcine model of DCD compared to the current clinical standard with the DPP protocol. In addition, we have validated findings in rodent studies and shed new light on underlying mechanisms of HOPE in the heart. Nonetheless, further investigation to define the optimal duration and precise conditions of HOPE is required to ensure maximal cardioprotection. This work comprises a substantial advancement in the translation of HOPE from the domain of fundamental research towards a clinical context. Importantly, our approach does not replace NMP as a preservation/graft storage strategy with HOPE, but rather implements hypothermic perfusion as a brief post-procurement, cardioprotective intervention, which may permit the combination of HOPE's beneficial effects and the

state-of-the-art evaluation of grafts in a beating state prior to transplantation, representing a key advancement in the field.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Conflict of interest: none declared.

DATA AVAILABILITY

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

Author contributions

Manuel Egle: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Visualization; Writing—original draft. **Adrian Segiser:** Conceptualization; Formal analysis; Investigation; Methodology; Writing—review & editing. **Alexia Clavier:** Investigation; Writing—review & editing. **Georgia Beer:** Investigation; Writing—review & editing. **Anja Helmer:** Investigation; Writing—review & editing. **Rahel Ottersberg:** Investigation; Writing—review & editing. **Selianne Graf:** Investigation; Methodology; Writing—review & editing. **Maria Arnold:** Formal analysis; Writing—review & editing. **Fabio Zulauf:** Methodology; Writing—review & editing. **Deborah Lagger:** Investigation; Writing—review & editing. **Maris Bartkevics:** Investigation; Writing—review & editing. **Alexander Kadner:** Investigation; Methodology; Supervision; Writing—review & editing. **Daja Krummenacher:** Investigation; Methodology; Writing—review & editing. **Peter Vermathen:** Investigation; Methodology; Writing—review & editing. **Matthias Siepe:** Conceptualization; Funding acquisition; Resources; Writing—review & editing. **Sarah Longnus:** Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing—review & editing.

Reviewer information

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