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ORIGINAL PRE-CLINICAL SCIENCE

Donor heart ischemic time can be extended beyond 9 hours using hypothermic machine perfusion in sheep

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Abbreviations: SCS, Static cold storage; PGD, primary graft dysfunction; HMP, hypothermic machine perfusion; BD, brain death; HTx, heart transplantation; CPB, cardiopulmonary bypass; EDTA, Ethylenediaminetetraacetic acid; cTnI, cardiac troponin I; IL, interleukin; TNFa, tumor necrosis factor alpha; BET-1, big endothelin-1; NIHP, Non-ischemic heart preservation

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KEYWORDS:
heart transplantation;
hypothermic machine perfusion;
organ preservation;
static cold storage

BACKGROUND: The global shortage of donor hearts available for transplantation is a major problem for the treatment of end-stage heart failure. The ischemic time for donor hearts using traditional preservation by standard static cold storage (SCS) is limited to approximately 4 hours, beyond which the risk for primary graft dysfunction (PGD) significantly increases. Hypothermic machine perfusion (HMP) of donor hearts has been proposed to safely extend ischemic time without increasing the risk of PGD.
METHODS: Using our sheep model of 24 hours brain death (BD) followed by orthotopic heart transplantation (HTx), we examined post-transplant outcomes in recipients following donor heart preservation by HMP for 8 hours, compared to donor heart preservation for 2 hours by either SCS or HMP.
RESULTS: Following HTx, all HMP recipients (both 2 hours and 8 hours groups) survived to the end of the study (6 hours after transplantation and successful weaning from cardiopulmonary bypass), required less vasoactive support for hemodynamic stability, and exhibited superior metabolic, fluid status and inflammatory profiles compared to SCS recipients. Contractile function and cardiac damage (troponin I release and histological assessment) was comparable between groups.
CONCLUSIONS: Overall, compared to current clinical SCS, recipient outcomes following transplantation are not adversely impacted by extending HMP to 8 hours. These results have important implications for clinical transplantation where longer ischemic times may be required (e.g., complex surgical cases, transport across long distances). Additionally, HMP may allow safe preservation of "marginal" donor hearts that are more susceptible to myocardial injury and facilitate increased utilization of these hearts for transplantation.
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Heart transplantation (HTx) continues to be challenged by donor heart availability.[1] Preservation of donor hearts via static cold storage (SCS) has not changed in 50 years, however SCS beyond 4 hours increases the probability of primary graft dysfunction (PGD), the major driver of post-transplant morbidity and mortality.[1-3] Machine perfusion preservation of donor hearts as an alternative to SCS is gaining momentum. Normothermic machine perfusion has expanded the utilization of donation after circulatory death and marginal donor hearts.[4,5] Hypothermic machine perfusion (HMP) is now undergoing experimental and early limited clinical investigation.[6-8] Previous work suggests that HMP with a portable, semiautonomous system using a hyperoncotic, oxygenated cardioplegia can safely preserve brain dead (BD) pig hearts for 24 hours with acceptable cardiac function post-transplant.[7] However, the true translational potential of extended HMP is yet to be determined, as no study has yet compared post-transplant outcomes against SCS in a clinically-relevant setting.

To be adopted for clinical use, HMP must deliver, at a minimum, preservation beyond the currently accepted SCS "safe" ischemic limit, (beyond which the risk of PGD increases) with outcomes that are noninferior to SCS. If HMP can facilitate longer preservation than SCS without the risk of PGD, its use will increase donor heart utilization for situations such as a projected long ischemic time (geographical distance or recipient surgical complexity) that may have a prohibitive risk using SCS. Using our clinically relevant BD sheep model of orthotopic HTx,[9] we aimed to determine if post-transplant outcomes following 8 hours HMP preservation was non-inferior to 2 hours of SCS.

Material and methods

Experimental design

This study employed a sheep model of orthotopic HTx from BD or sham (non-BD) donors using previously published methods.[9] Donor

sheep were either exposed to 24 hours BD, or sham-operated (no neurological injury).[9-11] Following confirmation of donor BD (or sham), critical care management was provided for 24 hours. The donor heart was then procured and preserved by SCS (2 hours) or HMP (2 or 8 hours). A healthy recipient was then prepared, cardiopulmonary bypass (CPB) established, and the native heart removed. The preserved donor heart was implanted using the bicaval orthotopic HTx technique.[11] The recipient was then weaned from CPB and monitored for up to 6 hours (Figure 1A). Three experimental recipient groups were compared following HTx: 2 hours SCS (n = 16), 2 hours HMP (n = 12), and 8 hours HMP (n = 14) (Table S1). All procedures for donor and recipient preparation, heart procurement (SCS only by us,[9] HMP by others[7]), orthotopic HTx, and critical care management have been previously published[9] (see Supplemental Material). Eighty-four female merino cross-bred sheep (Ovis aries, first cross ewes, 1-3 years) were used. Animal ethics was approved by the QUT Animal Ethics Committee (AEC) (16000001109) and ratified by the University of Queensland AEC (QUT/393/17/QUT). All experiments were performed in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 8th Edition 2013* and the *Animal Care and Protection Act 2001 (QLD)*.

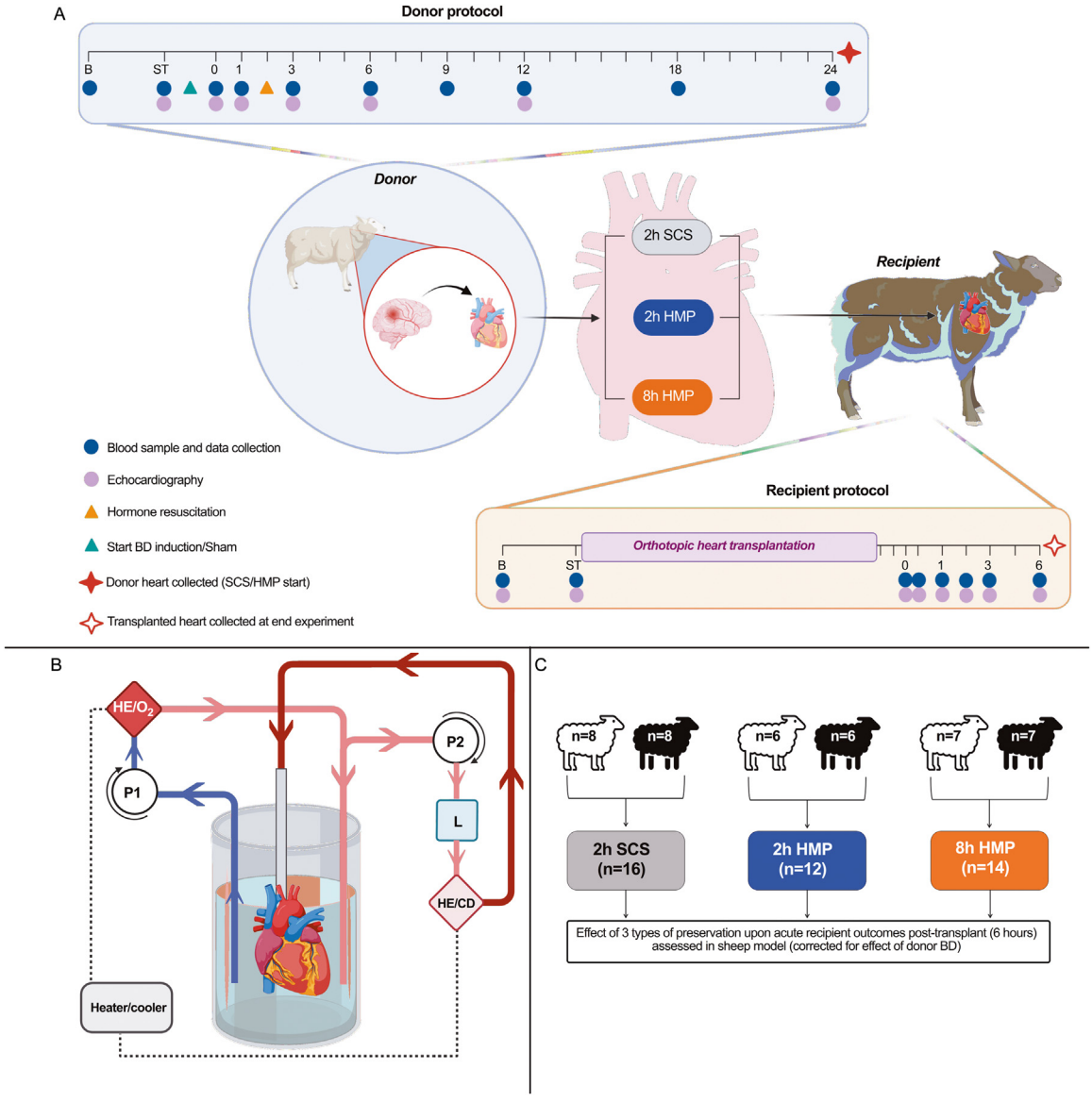

Figure 1 Hypothermic machine perfusion in sheep heart transplantation. (A) Donor animals were exposed to brain death (or sham injury), then monitored in critical care settings for 24 hours. Hearts were procured and preserved either by static cold storage (SCS) for 2 hours (2 hours SCS) or hypothermic machine perfusion for 2 or 8 hours (2 hours HMP or 8 hours HMP respectively). Donor hearts were then implanted into healthy recipients via orthotopic heart transplantation, weaned form cardiopulmonary bypass, and recipients monitored for a further 6 hours. (B), For constant oxygenation and cooling of the solution, Pump 1 (P1, recirculation line) removes HMP perfusion solution from the reservoir and delivers it to the oxygenator (HE/O_2) that is connected to the heater cooler. Solution leaves the oxygenator and is returned to the reservoir. Pump 2 (P2) removes solution from the recirculation line and delivers it to the leukocyte filter (L), followed by the cardioplegia delivery system (HE/CD), connected to the heater cooler, forming a cooling circuit with the oxygenator). From HE/CD, solution is delivered to the heart via the cannulated ascending aorta. The heart cannula is secured to the preservation system, and the heart submerged in preservation solution. (C), Grouping of donor sham (white sheep) and brain dead (black sheep) animals for study analysis. (A) and (B) illustrations created with BioRender.com.

Donor brain death and heart preservation

Using our established model,[9-11] BD was induced by inflating a Foley catheter placed in the brain. For sham donors, the Foley catheter was inserted but not inflated, and animals rested for 30 min.[9,11] All donors were monitored for 24 hours following confirmation of BD/sham. Donors were then prepared for heart procurement,[9] and donor hearts were preserved by 2 hours SCS, 2 hours HMP, or 8 hours HMP (Table S1) as per below:

SCS

Ice-cold St Thomas's cardioplegia was infused into the aortic root (20 ml/kg). Following explantation, the donor heart was preserved in 1-liter ice-cold St Thomas's cardioplegia, double-bagged in ice slush, and the organ bag placed in an ice cooler containing ice slush.[9]

HMP

The HMP system used was a modified laboratory-grade version of that previously published by Steen et al[7] (Figure 1B and C). See Supplemental Material for preparation of the HMP system and preservation solution. To arrest the donor heart, HMP preservation solution was infused into the aortic root via a line connected to the HMP system, using a perfusion pressure of 60 mm Hg (approximately 500 ml of oxygenated HMP solution delivered at 8˚C). Following explantation, a self-deairing cannula was inserted into the aorta which was ligated around the cannula, and the mitral valve made insufficient with a silastic tube placed across the mitral valve.[7] The heart was then submerged in the cooled preservation solution within the HMP reservoir, the aortic cannula was connected to the perfusion system and deaired. Antegrade coronary perfusion was initiated within 5 mins of heart explantation. The heart remained in the reservoir for the duration of preservation (2 or 8 hours), maintained at 8˚C. The heart was intermittently perfused in cycles of 15 min perfusion (using a perfusion pressure of 20-25 mm Hg) and 60 mins of nonperfusion.[7] More detailed methods describing donor preparation, procedures, heart retrieval and HMP machine preparation are outlined in the Supplemental Material.

Orthotopic heart transplantation

Recipients underwent orthotopic HTx using our established model,[9] and all donor heart retrievals and implantations were performed by the same, significantly experienced, senior cardiothoracic transplantation surgeon. Following establishment of cardiopulmonary bypass (CPB), the native recipient heart was removed, and the donor heart transplanted orthotopically.[9] Following completion of anastomoses and deairing, the aortic cross-clamp was removed. The cardiac allograft underwent 30 min reperfusion, before attempting to wean from CPB. Recipient animals were observed for 6 additional hours following successful separation from CPB. Animals were humanely euthanized at study completion using pentobarbitone (0.5 ml/kg). Following euthanasia, all hearts were immediately retrieved and preserved in ice-cold Krebs buffer as previously described.[11] More detailed methods describing orthotopic heart transplantation and post-transplant monitoring in recipients are provided in the Supplemental Material.

Data and sample collection

In donors and recipients, hemodynamics, ventilation, blood gases, and fluids were recorded, arterial blood collected, and epicardial echocardiography performed at designated timepoints (Figure 1A, also Supplemental Material).[9] During HMP, perfusate samples were collected at the end of designated 15 min perfusion cycles. Whole blood (EDTA) and plasma samples were assessed for hematological profiles, biochemistry, inflammatory cytokines and cardiac/endothelial injury biomarkers using previously published methods.[9,10,12-16] Formalin-fixed left and right ventricular samples of each heart were prepared and scored by an independent, blinded, specialist veterinary pathologist (Table S2). Cardiac edema was determined by measuring the wet-to-dry weights of left and right ventricular samples. More detailed methods regarding data and sample collection and processing are outlined in the Supplemental Material.

Statistical analysis

Sheep were randomized into 6 study groups through random numbers generated using block randomization with SAS version 9.4, assuming a fixed block length of 10. The 6 groups represented the combination of donor type (BD, Sham) and preservation method (2 hours SCS, 2 hours HMP, 8 hours HMP).

Statistical analysis examined changes in measured clinical parameters over time and between preservation methods. Each clinical parameter was analysed using a linear mixed-effects model.[17,18] Preservation method and donor type were included as categorical fixed effects, with SCS and Sham defined as the reference level, respectively. Time in hours was specified as a continuous fixed effect. The interaction between time and preservation method was also included. A random effect was specified for each sheep to account for within-subject correlation from repeated measurements over time. Model assumptions were assessed using QQ and residual plots. Fixed effects were reported as estimates with 95% confidence intervals (CI) and *p*-values were estimated using Sattherwaite's method.

A Kruskal-Wallis test was used to compare total ischemic, ex situ (preservation) and bypass times (data listed in Results), and wet/dry weights. All hypothesis testing is two-tailed and a *p*-value less than 0.05 was considered statistically significant. All statistical analyses were performed with R Version 4.0.5.

Results

Study population

Forty-two HTx experiments were performed (2 hours SCS: 16 experiments, 2 hours HMP: 12 experiments, 8 hours HMP: 14 experiments). Within each preservation group, an equal number of donor hearts were retrieved from either sham or BD donors (Table S1). One SCS recipient was excluded from further analysis as it ended the study early due to a technical bleeding issue. Donor and recipient baseline parameters did not differ between groups (Table S3 and S4 respectively).

Recipient post-transplant survival and ischemic times

Following HTx (Figure 1A), all recipients were successfully weaned from CPB. All HMP recipients ($n = 26$) completed the study to 6 hours observation post-transplant, regardless of preservation time or donor injury. However, 31% (5/16) of SCS recipients did not complete the study.

Table 1 Linear Mixed-Effect Model for 2 hours SCS, 2 hours HMP or 8 hours HMP Recipients Post-transplant

Comparison	Group	Estimates (95% CI), *p* value
	Arterial lactate (mmol/liter)	
Preservation group	SCS	Reference
	2 hours HMP	-2.00 (-3.86 to -0.13), 0.047
	8 hours HMP	-0.52 (-2.30 to 1.27), 0.585
Time, hours		0.76 (0.61 to 0.92), <0.001
Donor injury	Sham	Reference
	BD	0.26 (-1.17 to 1.69), 0.730
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-0.47 (-0.69 to -0.25), <0.001
	8 hours HMP	-0.45 (-0.66 to 0.24), <0.001
	Vasopressor dependency index (VDI, mm Hg^{-1})	
Preservation group	SCS	Reference
	2 hours HMP	-0.36 (-0.77 to 0.049), 0.1
	8 hours HMP	-0.32 (-0.71 to 0.072), 0.13
Time, hours		0.15 (0.11-0.19), <0.001
Donor injury	Sham	Reference
	BD	-0.09 (-0.40 to 0.21), 0.57
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-0.09 (-0.15 to -0.03), 0.002
	8 hours HMP	-0.11 (-0.16 to -0.05), 0.003
	Arterial base excess (Ecf)	
Preservation group	SCS	Reference
	2 hours HMP	5.93 (3.68-8.18), <0.001
	8 hours HMP	5.21 (3.05-7.37), <0.001
Time, hours		-0.49 (-0.72 to -0.28), <0.001
Donor injury	Sham	Reference
	BD	-1.55 (-3.26 to 0.16), 0.09
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	0.26 (-0.039 to 0.57), 0.09
	8 hours HMP	0.16 (-0.14 to 0.45), 0.29
	Urine output (ml/kg/hour)	
Preservation group	SCS	Reference
	2 hours HMP	0.85 (-0.02 to 1.72), 0.065
	8 hours HMP	0.52 (-0.32 to 1.35), 0.24
Time, hours		-0.01 (-0.16 to 0.14), 0.88
Donor injury	Sham	Reference
	BD	-0.41 (-0.92 to 0.10), 0.13
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	0.16 (-0.07 to 0.38), 0.18
	8 hours HMP	0.20 (-0.02 to 0.42), 0.077
	Cumulative fluid balance (L)	
Preservation group	SCS	Reference
	2 hours HMP	-1.59 (-3.0 to -0.23), 0.031
	8 hours HMP	-1.74 (-3.05 to -0.43), 0.015
Time, hours		0.50 (0.38-0.62), <0.001
Donor injury	Sham	Reference
	BD	-0.42 (-1.43 to 0.60), 0.44
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-0.18 (-0.29 to 0.05), 0.18
	8 hours HMP	-0.17 (-0.33 to -0.002), 0.049
	Fractional area change (FAC, %)	
Preservation group	SCS	Reference
	2 hours HMP	4.32 (-5.12 to 13.81), 0.39
	8 hours HMP	2.38 (-6.62 to 11.39), 0.62
Time, hours		-0.02 (-1.26 to 1.25), 0.98
Donor injury	Sham	Reference
	BD	1.97 (-4.01 to 7.96), 0.53

(*continued on next page*)

Table 1 (*Continued*)

Comparison	Group	Estimates (95% CI), *p* value
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	1.03 (-0.80 to 2.83), 0.28
	8 hours HMP	0.62 (-1.12 to 2.34), 0.49
	Global radial strain (GRS, %)	
Preservation group	SCS	Reference
	2 hours HMP	-0.61 (-14.0 to 12.73), 0.93
	8 hours HMP	8.59 (-4.04 to 21.22), 0.20
Time, hours		-0.05 (-2.23 to 2.14), 0.97
Donor injury	Sham	Reference
	BD	0.086 (-6.72 to 6.89), 0.98
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	1.16 (-2.00 to 4.34), 0.48
	8 hours HMP	1.15 (-1.87 to 4.16), 0.46
	Cardiac troponin I (cTnI, ng/ml)	
Preservation group	SCS	Reference
	2 hours HMP	5.66 (-17.19 to 28.51), 0.64
	8 hours HMP	7.49 (-14.57 to 29.55), 0.52
Time, hours		2.17 (0.94-3.40), <0.001
Donor injury	Sham	Reference
	BD	-4.61 (-22.29 to 13.06), 0.62
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-0.41 (-2.11 to 1.30), 0.64
	8 hours HMP	0.18 (-1.46 to 1.83), 0.83
	Creatine kinase (IU/liter)	
Preservation group	SCS	Reference
	2 hours HMP	-2067 (-5131 to 997), 0.21
	8 hours HMP	-2577 (-5522 to 367), 0.10
Time, hours		163 (55-271), 0.003
Donor injury	Sham	Reference
	BD	-963 (-3410 to 1484), 0.46
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	41 (-109 to 192), 0.60
	8 hours HMP	-50 (-196 to 98), 0.51
	Interleukin-6 (IL-6, pg/ml)	
Preservation group	SCS	Reference
	2 hours HMP	-41,368 (-104,822 to 22,087), 0.22
	8 hours HMP	-32,285 (-93,287 to 28,717), 0.32
Time, hours		1,2425 (7949-16,930), <0.001
Donor injury	Sham	Reference
	BD	13,451 (-36,033 to 62,936), 0.61
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-11,983 (-18,272 to -5722), <0.001
	8 hours HMP	-12,159 (-18,240 to -6107), <0.001
	Interleukin-8 (IL-8, pg/ml)	
Preservation group	SCS	Reference
	2 hours HMP	-729 (-1272 to -187), 0.014
	8 hours HMP	-517 (-1039 to 5), <0.001
Time, hours		-160 (-213 to -106), <0.001
Donor injury	Sham	Reference
	BD	24 (-386 to 435), 0.91
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	90 (15-165), 0.02
	8 hours HMP	33 (-39 to 105), 0.37
	Big endothelin-1 (BET-1, pg/ml)	
Preservation group	SCS	Reference
	2 hours HMP	-0.82 (-2.48 to 0.83), 0.35
	8 hours HMP	-1.31 (-2.90 to 0.28), 0.13
Time, hours		-0.03 (-0.09 to 0.02), 0.26
Donor injury	Sham	Reference
	BD	-0.30 (-1.62 to 1.02), 0.67

(*continued on next page*)

Table 1 (*Continued*)

Comparison	Group	Estimates (95% CI), *p* value
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-0.05 (-0.13 to 0.03), 0.23
	8 hours HMP	-0.02 (-0.09 to 0.06), 0.67
	Hyaluronan (ng/ml)	
Preservation group	SCS	Reference
	2 hours HMP	-548 (-1924 to 828), 0.45
	8 hours HMP	-580 (-1905 to 746), 0.41
Time, hours		272 (133-411), <0.001
Donor injury	Sham	Reference
	BD	-393 (-1425 to 639), 0.47
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-336 (-529 to -143), <0.001
	8 hours HMP	7 (-180 to 194), 0.94
	Interleukin-10 (IL-10, pg/ml)	
Preservation group	SCS	Reference
	2 hours HMP	-1232 (-5825 to 3360), 0.61
	8 hours HMP	-1852 (-6271 to 2567), 0.43
Time, hours		-1116 (-1603 to -627), <0.001
Donor injury	Sham	Reference
	BD	-3645 (-7080 to -210), 0.051
Interaction between preservation group and time	SCS	Reference
	2 hours HMP	-685 (-1368 to -2), 0.052
	8 hours HMP	-103 (-763 to 557), 0.76

BD, brain death; HMP, hypothermic machine perfusion; SCS, static cold storage.

Statistical analysis examined changes in measured clinical parameters over time and between preservation methods. Each clinical parameter was analyzed using a linear mixed-effects model. Preservation method and donor type were included as categorical fixed effects, with SCS and Sham defined as the reference level, respectively. Time in hours was specified as a continuous fixed effect. The interaction between time and preservation method was included. A random effect was specified for each sheep to account for within-subject correlation from repeated measurements over time. Data in Table 1 reports the fixed effects as estimates with 95% confidence intervals (CI), and reported p-values were estimated using Sattherwaite's method. Sample sizes were as follows: 2 hours SCS: $n = 15$, 2 hours HMP: $n = 12$, and 8 hours HMP: $n = 14$.

Specifically, 1 SCS recipient died 20 mins following separation from CPB, 3 were terminated 2 to 3 hours post-transplant as they became unresponsive to maximal vasopressor support, and 1 recipient was terminated due to a technical bleeding issue (noted above). Donor heart ischemic (Data expressed as mean ± SD; 2 hours SCS: 175 ± 28 mins; 2 hours HMP: 200 ± 44 mins; 8 hours HMP: 576 ± 18 mins) and ex situ (Data expressed as mean ± SD; 2 hours SCS: 104 ± 20 mins; 2 hours HMP: 123 ± 44 mins; 8 hours HMP: 491 ± 9 mins) preservation times were no different between the 2 hours preservation groups (2 hours SCS vs 2 hours HMP ischemic time: $p = 0.10$; ex situ time: $p = 0.17$), and within the usual range for clinical HTx.[2] The time recipients spent on CPB remained consistent (Data expressed as mean ± SD; 2 hours SCS: 138 ± 43 mins; 2 hours HMP: 119 ± 22 mins; 8 hours HMP: 131 ± 32 mins; $p = 0.59$).

Recipient hemodynamic, metabolic, and fluid status profiles

HMP recipients (2 hours and 8 hours) required significantly less vasoactive support compared to SCS recipients to maintain stable hemodynamic function (Table 1 and Figure 2A, for interaction between preservation group and time, $p = 0.002$ for 2 hours HMP, and $p = 0.003$ for 8 hours HMP). Recipient urine output was generally lower in the SCS group, but no statistically significant differences were detected (Table 1 and Figure 2B). However, HMP recipients (both 2 hours and 8 hours) required less fluid volume post-transplant compared to SCS recipients (Table 1 and Figure 2C, $p < 0.05$ for 8 hours HMP vs SCS over time). Blood lactate levels rose over time in all groups (Table 1 and Figure 2D, $p < 0.001$), however compared to SCS, both 2 hours HMP and 8 hours HMP recipients demonstrated significantly lower levels of blood lactate post-transplant (for interaction between preservation group and time, $p < 0.001$ for 2 hours HMP and 8 hours HMP respectively). The arterial base excess decreased over time ($p < 0.001$) but no significant differences were detected between preservation groups over time (Table 1 and Figure 2E). There was no effect of donor injury (BD or sham) upon recipient hemodynamic, metabolic and fluid status profiles between the 3 preservation groups (Table 1).

Cardiac function and injury

Donor heart contractile function was comparable between groups prior to induction of donor BD/sham (Figure 2F and G, Table S3). Following HTx, fractional area change

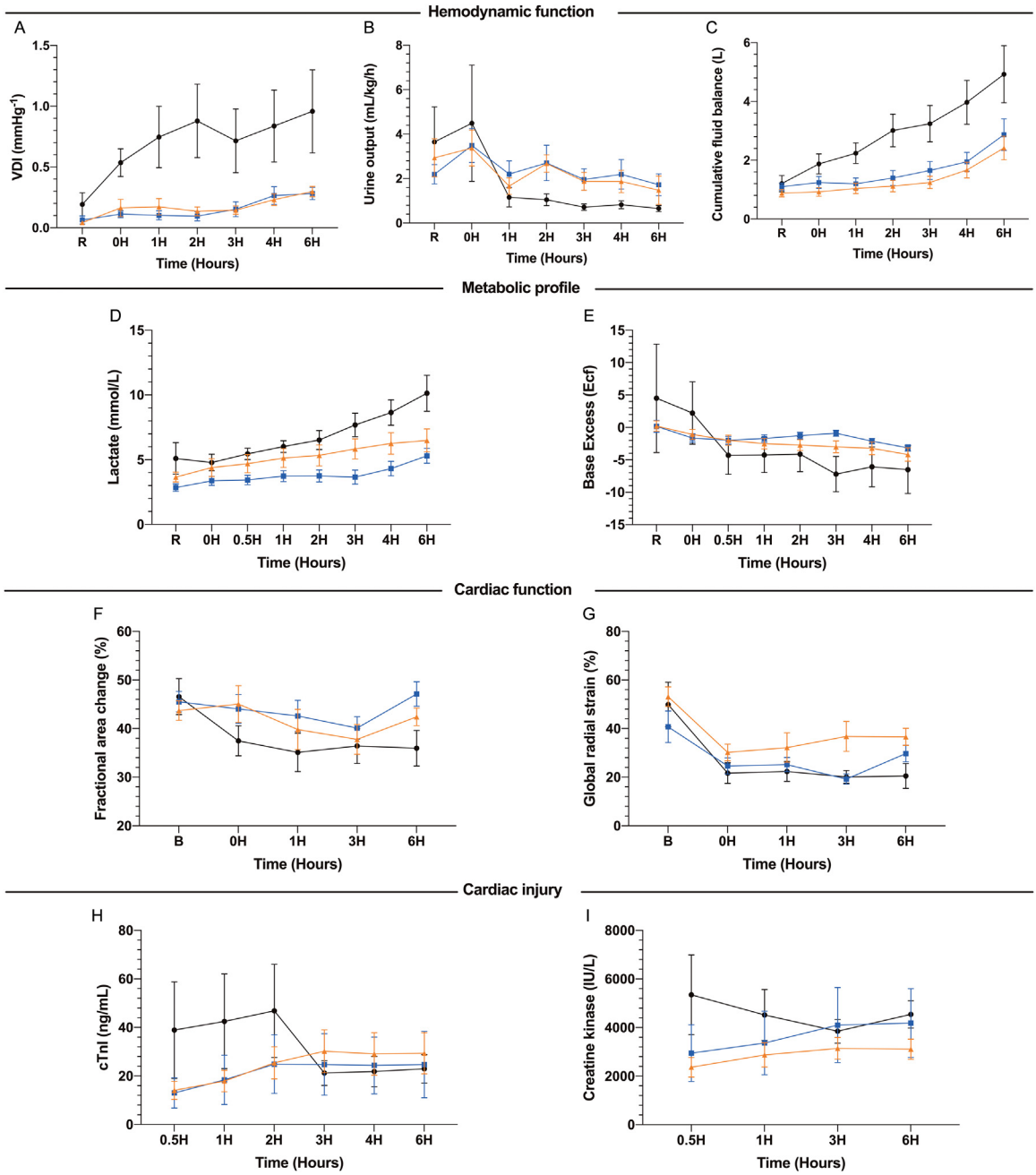

Figure 2 Recipient hemodynamic function, metabolic profile and cardiac function post-transplant.

Hearts were retrieved following 24 hours monitoring in BD/sham donors and preserved by 2 hours SCS (total n = 15; sham n = 8; BD n = 7), 2 hours HMP (total n = 12; sham n = 6, BD n = 6), or 8 hours HMP (total n = 14; sham n = 7, BD n = 7). Following preservation, donor hearts were implanted into recipients via standard orthotopic heart transplantation, after which donor hearts were reperfused (R), recipients were weaned from cardiopulmonary bypass (0H) and monitored for up to 6 additional hours (0.5H-6H). Group differences in recipient hemodynamic function and metabolic profile were determined by (A) vasopressor dependency index (VDI, mm Hg^{-1}), (B) urine output (ml/kg/h), (C) cumulative fluid balance (L), (D) blood lactate (mmol/liter) and (E) base excess (Ecf). Changes in cardiac function were determined using two-dimensional echocardiography to determine (F) fractional area change (%) and (G) global radial strain (%). Cardiac damage was assessed by (H) cardiac troponin I (cTnI, ng/ml) and (I) creatine kinase (IU/liter) levels in recipient plasma collected serially post-transplant. B—Baseline (in F and G - baseline function of donor heart in donor, measured 1 hour following completion of instrumentation procedures in donor). Statistical analysis examined changes in measured clinical parameters over time and between preservation methods using a linear mixed-effects model (Table 1). Symbols and lines represent mean ± S.E.M. Black dots = SCS, blue squares = 2 hours HMP, orange triangles = 8 hours HMP. Please refer to Figure S1 (Supplemental Material) for individual data per group over time.

(Figure 2F and Table 1) and global radial strain (Figure 2G and Table 1) were similar between groups. Plasma cardiac troponin I (cTnI) changed over time post-transplant ($p <$ 0.001) but was not different between groups over time (Table 1, Figure 2H). Approximately 2 to 3 hours post-transplant, HMP recipient cTnI expression stabilized (2 and 8 hours groups), and SCS recipient cTnI expression declined. No differences between groups over time were

observed in creatine kinase levels post-transplant (Figure 2I and Table 1). Similarly, donor injury (BD or sham) did not affect post-transplant cardiac function or injury biomarker expression (cTnI or CK) between preservation groups (Table 1).

Cardiac allograft histological assessment

Histological scoring post-transplant revealed injury across all groups (Figures 3 and 4, and S1), characterized by contraction band necrosis, myocytolysis, neutrophil infiltration and vascular damage (degeneration and hemorrhage of the vascular wall). Despite the variable injury observed, in comparison to SCS grafts, a minor degree of reduced myocytolysis (both 2 and 8 hours, Figure 4B) and neutrophil infiltration (2 hours only) was detected in HMP grafts (Figure 4D). There were no obvious ventricular differences within each heart (Figure 4). Comparison of cardiac wet/dry weight ratios suggest no differences in cardia edema between groups (Figure 4G).

Inflammatory/injury biomarkers

Levels of interleukin (IL)-6, lactate, and cTnI rose over time in HMP perfusate, with variable expression of white blood cells (Figure 5A-D). Post-transplant, HMP recipient systemic IL-6 expression (both 2 and 8 hours) was low compared to SCS recipients (Figure 5E and Table 1, $p < 0.001$ for effect of time, and effect of preservation over time). Systemic IL-8 and IL-10 expression generally reduced over time post-transplant within each group (Figure 5F and G, Table 1, $p < 0.001$ for effect of time), and no significant differences were detected between groups over time for IL-10 or BET-1 (Figure 5H). IL-8 was significantly lower in 2 hours HMP versus SCS over time (p 0.02, Table 1). Hyaluronan (Figure 5I) was relatively unchanged over time in 2 hours HMP recipients but increased in both SCS and 8 hours HMP groups. Expression levels were statistically significant between 2 hours HMP versus SCS over time ($p < 0.001$; Table 1).

Discussion

This study demonstrated that BD hearts can be safely preserved by HMP for 8 hours, orthotopically transplanted without developing PGD, with outcomes comparable to, or better than SCS. This is evidenced by favorable survival post-transplant, hemodynamic, metabolic and fluid profiles, preserved cardiac function, comparable histological cardiac injury and reduced systemic inflammation 6 hours post-transplant following HMP preservation.

Post-transplant hemodynamic function is a critical indicator of early HTx success. Poor hemodynamic function due to PGD requiring high inotrope and mechanical circulatory support in clinical HTx is associated with an increased risk of mortality,[19,20] acute kidney injury,[1,19] and ICU length of stay.[21] HMP recipients required less hemodynamic support, and displayed lower lactate levels compared to SCS recipients, despite significantly increased preservation time (8 hours group). SCS recipients developed severe vasoplegia, reflected by greater inotrope requirements and early study termination for some SCS recipients unresponsive to maximal support. Lower vasoactive use,[19,20,22] reduced lactate,[22-24] and improved fluid balance[25] in HMP recipients would likely translate into reduced morbidity and mortality in human HTx. Achieving these post-transplant outcomes, whilst simultaneously safely extending the ischemic time, is particularly important to overcome time and geographical distance barriers in clinical HTx.

Optimal cardiac allograft function is critical to HTx success, and contractile dysfunction is key to PGD diagnosis.[1,26,27] Despite extending HMP preservation time by 400%, heart function and extent of injury did not decline compared to SCS, which was coupled with greater recipient hemodynamic stability and superior survival. PGD pathology remains somewhat ill-defined, partly due to interacting donor, procedural and recipient factors that influence its incidence.[1,23,28] Importantly, BD modifies myocardial architecture through calcium overload in vascular smooth muscle, and via development of myocytolysis, contraction bands and coagulative necrosis in response to catecholamine excess.[29-32] Despite this, significant cardiac ultrastructural derangements do not necessarily translate into early graft failure in humans.[29] Other HMP studies report preserved myocardial and endothelial cell structures following reperfusion, with variable outcomes regarding the extent of cardiac edema,[8,33,34] which is a common concern for perfusion preservation systems.[35,36] However, cardiac edema was no different between groups in our study, potentially reflecting an effect of the hyperoncotic cardioplegia used for HMP.[7,37]

HMP reportedly prevents accumulation of metabolic and inflammatory waste.[8,38-40] We and others[8] have detected progressive biomarker elevations in HMP perfusate, though the absence of an SCS comparison, and sample collection from the machine reservoir (e.g., vs coronary sinus), makes interpretation difficult. Additionally, reduced inflammation post-transplant both systemically (predominantly IL-6) and in cardiac tissue (trend for reduced neutrophil infiltration) following HMP preservation was observed. IL-6 has been consistently linked with cardiac allograft rejection incidence and severity in human HTx recipients.[41] In animals, myocardial IL-6 deficiency,[42] co-stimulatory blockade,[43] or neutralisation of IL-6[44] improves graft survival, delays onset of rejection, and limits inflammatory cell infiltration. Thus, reducing the cardiac allograft inflammatory milieu during HMP may decrease or delay allograft rejection. While we and others[8] observe that HMP may limit HTx-mediated inflammation, the mechanism underlying this observation and its impact upon the incidence and severity of allograft rejection warrants further exploration.

Perfusion technologies may increase the donor heart pool, limit allograft injury, and safely increase preservation time. While different centers are gaining experience with

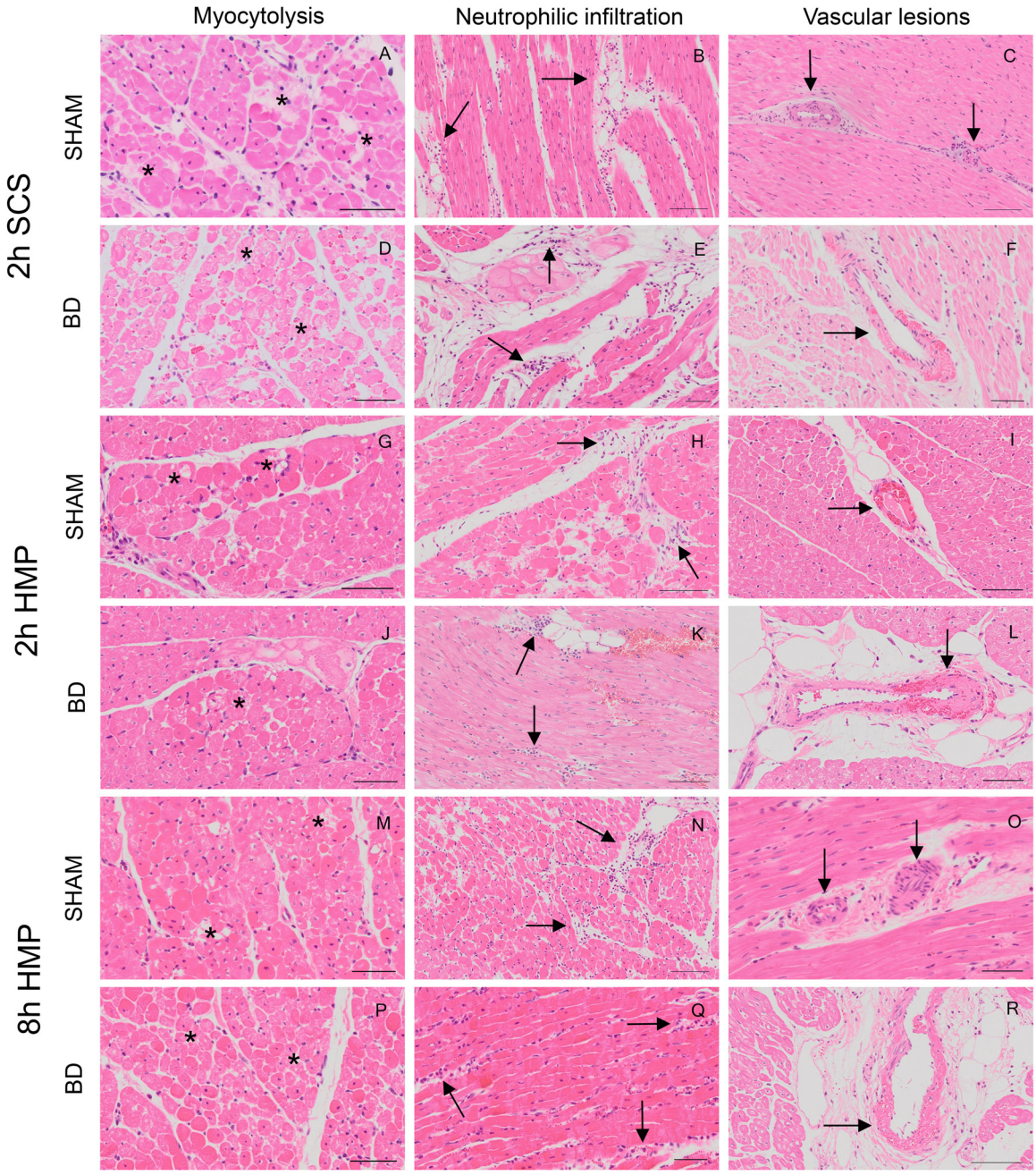

Figure 3 Representative histological images from hearts collected post-transplant across all groups depicting regions of myocytolysis, neutrophilic infiltration and vascular lesions. Images represent the following (scales indicated in μm for each panel): (A) mild rarefaction and vacuolation of myocardiocytes (*) 50 μm, (B) infiltration of low number of neutrophils in the interstitial tissue (arrows) 100 μm, (C) normal vascular wall of small arteries (arrows) 100 μm, (D) locally extensive area with mild degenerative changes (*) 50 μm, (E) low number of neutrophils infiltrating the interstitium (arrows) 50 μm, (F) degeneration (hypereosinophilia) and hemorrhage of the vascular wall (arrow) 50 μm, (G) mild vacuolation of myocardiocytes (*) 50 μm, (H) mild infiltration of neutrophils within a necrotic area (arrows) 100 μm, (I) hemorrhage of the vascular wall of a small artery (arrow) 50 μm, (J) focal area of vacuolation and cytoplasmic rarefaction of myocardiocytes (*) 50 μm, (K) multifocal infiltration of neutrophils within the interstitium (arrows) 100 μm, (L) focal degeneration and hemorrhage of the vascular wall (arrow) 50 μm, (M) locally extensive area of myocytolysis (*) 50 μm, (N) infiltration of moderate number of neutrophils within the interstitium (arrows) 100 μm, (O) normal vascular wall of 2 small arteries (arrows) 50 μm, (P) locally extensive area of myocardiocytes vacuolation (*) 50 μm, (Q) neutrophilic infiltration of the interstitium (arrows) 50 μm, (R) degeneration and hypereosinophilia of the vascular wall (arrow) 100 μm. Abbreviations: BSD, brain death; HMP, hypothermic machine perfusion; SH, sham; SCS, static cold storage. Please refer to Figure S2 (Supplemental Material) for representative histological images depicting regions of contraction band necrosis, necrosis and hemorrhage.

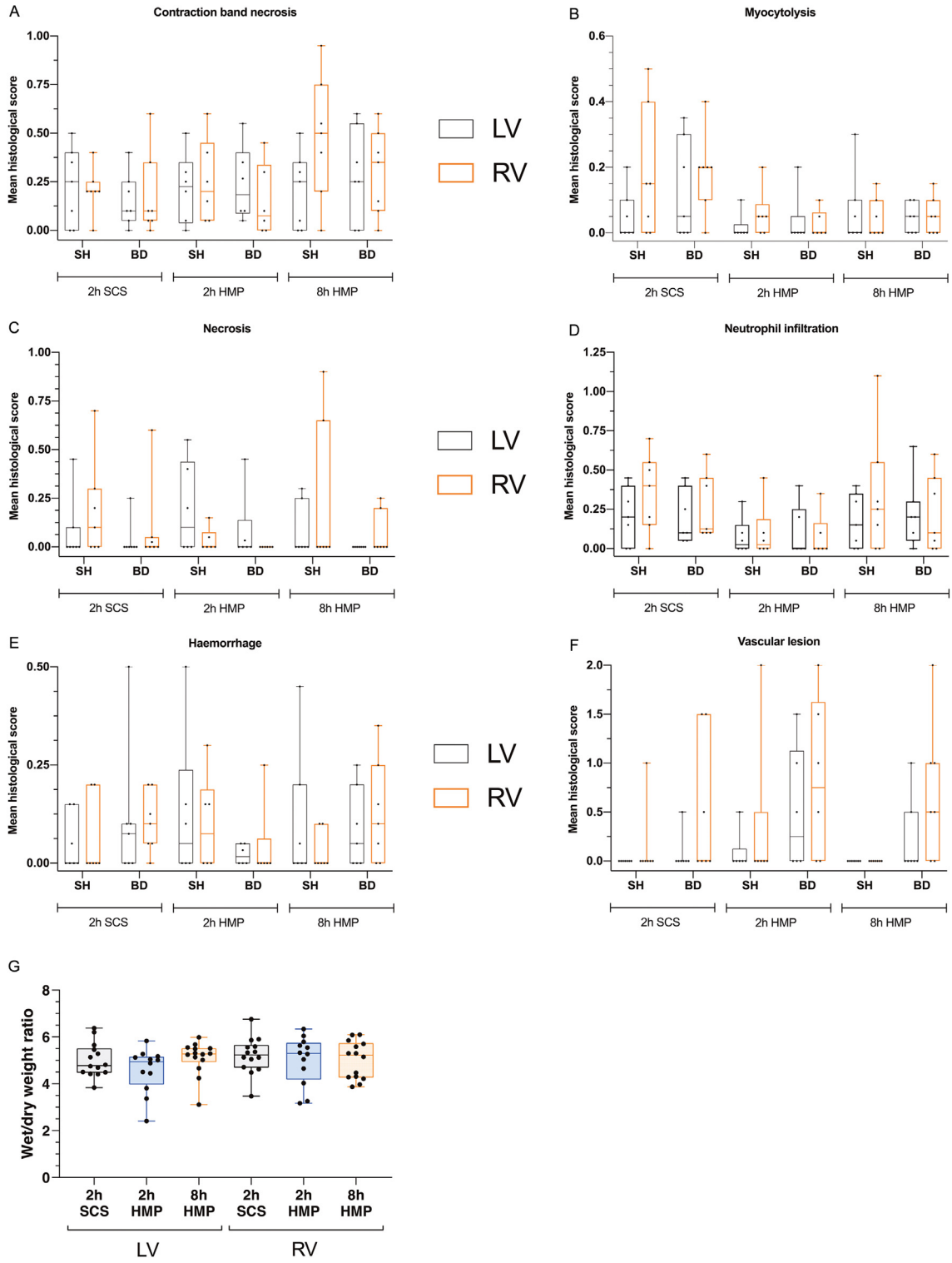

Figure 4 Cardiac allograft histological scores and edema. A-F (minimum to maximum) depict individual histological scores (black dots) or each ventricle per heart (left ventricle − LV, black boxes; right ventricle − RV, orange boxes), exposure to donor brain death (BD) or sham (SH), and preservation strategy. No statistical analysis was performed, and sample sizes are as follows: SH 2 hours SCS $n = 7$ (Data missing for $n = 1$ as sample not collected), BD 2 hours SCS $n = 7$ ($n = 1$ excluded due to technical error as outlined in results), SH 2 hours HMP $n = 6$, BSD 2 hours HMP $n = 6$, SH 8 hours HMP $n = 7$, BSD 8 hours HMP $n = 7$. (G) (minimum to maximum) depicts allograft wet-to-dry weight ratios, black dots represent individual samples, separated by left and right ventricle. For data in G, a Kruskal-Wallis test was used to compare group differences. Sample sizes in J are as follows, SCS: $n = 13$ (SH $n = 6$, BD $n = 7$), 2 hours HMP: $n = 12$ (SH $n = 6$, BD $n = 6$), 8 hours HMP: $n = 14$ (SH $n = 7$, BD $n = 7$). Data missing for $n = 2$ (SH 2 hours SCS) due to sample processing and collection errors. Black box = SCS, blue box = 2 hours HMP, orange box = 8 hours HMP.

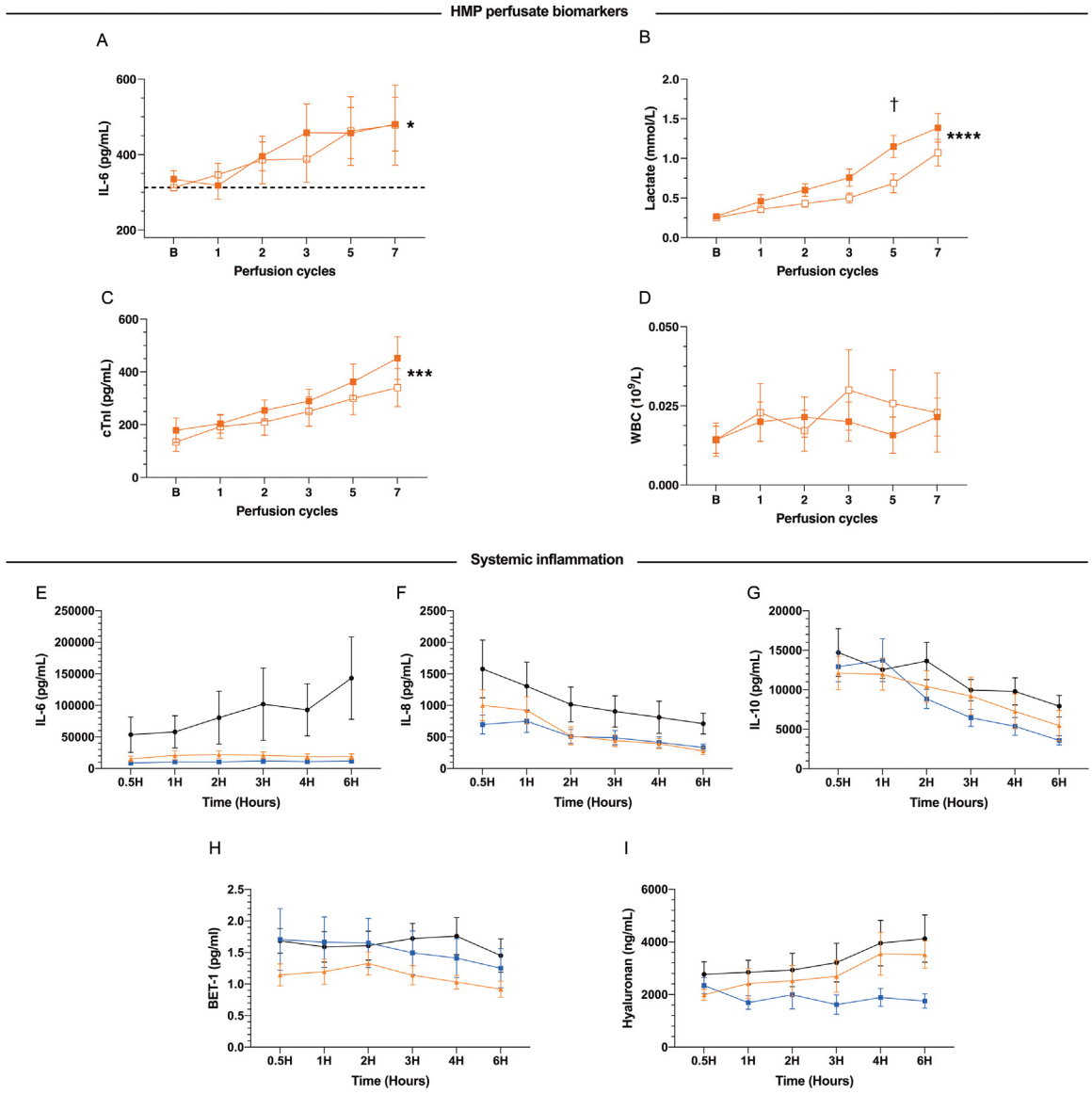

Figure 5 HMP perfusate biomarkers and systemic inflammation post-transplant.

Perfusate during 8 hours HMP was collected from baseline (B, no heart on system) up to 7 cycles of perfusion preservation by HMP, to detect (A) IL-6, (B) lactate, (C) cardiac troponin I (cTnI), and (D) total white blood cell count (WBC). For (A-D), data separated by donor heart exposure to brain death (full square, $n = 7$) or sham (open square, $n = 7$). Following successful weaning from cardiopulmonary bypass (0H), changes in recipient plasma (E) interleukin-6 (IL-6), (F) interleukin-8 (IL-8), (G) interleukin-10 (IL-10); (H) big endothelin-1 (BET-1), and (I) hyaluronan post-transplant (0.5H-6H). In E-G, black dots = SCS (total $n = 15$, sham $n = 8$, BD $n = 7$), blue squares = 2 hours HMP (total $n = 12$, sham $n = 6$, BD $n = 6$), orange triangles = 8 hours HMP (total $n = 14$; sham $n = 7$, BD $n = 7$). Data in A-D analyzed using a two-way repeated measure ANOVA (except for lactate, where a mixed-effect analysis was performed due to some missing values), for statistically significant differences detected over time: *, $p < 0.05$; ***, $p < 0.001$; **** $p < 0.0001$; differences between groups at a specific time: †, $p < 0.05$. For E-G, comparison of effect between groups over time was performed with linear mixed effect models (Table 1). Please refer to Figure S3 (Supplemental Material) for individual data per group over time.

perfusion approaches to heart preservation,[4-6,45,46] overarching clinical limitations persist (i.e., excessive costs, resources, legislative barriers). The Transmedics Organ Care System[1] is the first and only clinically available normothermic perfusion platform, and successfully expanded the donor pool by facilitating transplantation of marginal and "donation after circulatory death" hearts.[5] However, a recent study of BD heart transplants demonstrated that longer Transmedics preservation was a predictor of PGD.[47] Unlike the Transmedics system, the clinical version of the HMP system used in this study (XVIVO Heart Box System)[2] is simple to use, essentially autonomous, no cardiac physiology monitoring is required, and conversion to standard SCS in the event of machine failure safeguards the donor heart. Our study shows that HMP can safely extend preservation time without worsening outcomes

[1]Transmedics, Inc. Andover, MA, USA

[2]XVIVO Perfusion AB, Göteborg, Sweden.

post-transplant. Clinically, HMP may facilitate increased acceptance of donor hearts challenged by long ischemic times due to excessive geographical distances, or in cases of increased surgical complexity. Outcomes of this study formed part of the preclinical foundations for the Australian and New Zealand Non-Ischemic Heart Preservation (NIHP) trial, which is currently recruiting (ACTRN12620000595910p). This trial aims to determine the effect of 6 to 8 hours ischemic time using HMP preservation upon allograft function, the incidence of PGD and re-transplantation (up to 12 months). HMP is poised to overcome barriers to donor heart preservation that have remained unchanged for 50 years.

The experiments outlined are technically, logistically and financially challenging. We previously reported the challenges in developing large animal models of HTx that replicate clinical settings, and the paucity of models available for critical evaluation of novel therapies preceding clinical translation.[28] Despite this, our study was limited by the relatively short postoperative monitoring time in recipients (6 hours). Further, anatomical constraints in the ovine model associated with accessing the apex due to the short thoracotomy or sternotomy limited our ability to capture conventional apical views and perform wider echocardiographic analysis. Since PGD development in human HTx is invariably evident within 2 to 3 hours following CPB separation, we decided that 6 hours of observation would adequately capture PGD development. Extending the observation time may have revealed greater differences due to preservation strategy (e.g., in global cardiac function). However, particularly for SCS recipients, this would have likely required additional interventions beyond the scope of the study to achieve the desired aims (e.g., kidney dialysis, extracorporeal membrane oxygenation). Of note, the chest remained open during the follow up period. This could have impacted the fluid balance and hemodynamic stability of the animals[48,49] and this should be taken into account while interpreting our results. Our goal was not focused on understanding the mechanistic basis of the effects of HMP preservation in the donor heart, thus further studies are warranted to address this limitation. Additionally, studies were performed in young, healthy animals. Therefore, our results may not necessarily reflect the influence of impaired pathophysiology commonly observed in HTx recipients upon the observed clinical benefit of HMP.

The global shortage of donor hearts, geographical limitations to donor heart transportation and ischemic times, and the imperative to mitigate myocardial injury associated with BD and SCS requires consideration of alternative preservation strategies. We have demonstrated HMP can be used to safely extend preservation time to 8 hours without the penalty of PGD. These findings have implications for donor heart preservation strategies in jurisdictions where long ischemic times are required. Leaving the operating room with a well-functioning allograft and avoiding the consequences of mechanical circulatory support give patients the best chance of surviving HTx. HMP may be the key to this goal, as well as potentially expanding the availability of donor hearts.

Disclosures statement

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Author contributions

LS, GB, MP, DM, JF: Study conception and design. LS, GB, KW, MP, MB, K Sato, SH, CA, NB, EW, KH, KS, NG, TS, LB, CP, SP, CM, SC, MW, JR, HO, SL, GA, AH, JJ, NS, LJ, TH, NW, MR, JM, MM, PM, DP, JC, JS, DM, JF: Data collection. LS, GB, KW, MP, MB, K Sato, SH, CA, NB, EW, KH, NG, TS, LB, CP, SC, NW, DP, JC, JS, DM, JF: Data analysis. LS, DM: Writing − original draft preparation. LS, GB, KW, MP, MB, K Sato, SH, CA, NB, EW, KH, KS, NG, TS, LB, CP, SP, CM, SC, MW, JR, HO, SL, GA, AH, JJ, NS, LJ, TH, NW, MR, JM, MM, PM, DP, JC, JS, DM, JF: Writing − review, editing, and interpretation. LS, GB, KW, MP, MB, K Sato, SH, CA, NB, EW, KH, K Skeggs, NG, TS, LB, CP, SP, CM, SC, MW, JR, HO, SL, GA, AH, JJ, NS, LJ, TH, NW, MR, JM, MM, PM, DP, JC, JS, DM, JF: Final approval of submission.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.healun.2023.03.020.

References

1. Kobashigawa J, Zuckermann A, Macdonald P, et al. Report from a consensus conference on primary graft dysfunction after cardiac transplantation. J Heart Lung Transplant 2014;33:327-40.
2. Lund LH, Khush KK, Cherikh WS, et al. The Registry of the International Society for Heart and Lung Transplantation: thirty-fourth adult heart transplantation report-2017; focus theme: allograft ischemic time. J Heart Lung Transplant 2017;36:1037-46.
3. Russo MJ, Chen JM, Sorabella RA, et al. The effect of ischemic time on survival after heart transplantation varies by donor age: an analysis of the United Network for Organ Sharing database. J Thorac Cardiovasc Surg 2007;133:554-9.
4. Ardehali A, Esmailian F, Deng M, et al. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. Lancet 2015;385:2577-84.
5. Dhital KK, Iyer A, Connellan M, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. Lancet 2015;385:2585-91.
6. Nilsson J, Jernryd V, Qin G, et al. A nonrandomized open-label phase 2 trial of nonischemic heart preservation for human heart transplantation. Nat Commun 2020;11:2976.
7. Steen S, Paskevicius A, Liao Q, Sjoberg T. Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. Scand Cardiovasc J 2016;50:193-200.
8. Critchley WR, Stone JP, Liao Q, et al. Non-ischemic heart preservation via hypothermic cardioplegic perfusion induces immunodepletion of donor hearts resulting in diminished graft infiltration following transplantation. Front Immunol 2020;11:1621.
9. See Hoe LE, Wildi K, Obonyo NG, et al. A clinically relevant sheep model of orthotopic heart transplantation 24 h after donor brainstem death. Intensive Care Med Exp 2021;9:60.
10. Watts RP, Bilska I, Diab S, et al. Novel 24-h ovine model of brain death to study the profile of the endothelin axis during cardiopulmonary injury. Intensive Care Med Exp 2015;3:31.
11. Wells MA, See Hoe L, Molenaar P, et al. Compromised right ventricular contractility in an ovine model of heart transplantation following 24 hours donor brain stem death. Pharm Res 2021;169:105631.
12. Millar JE, Bartnikowski N, Passmore MR, et al. Combined mesenchymal stromal cell therapy and extracorporeal membrane oxygenation in acute respiratory distress syndrome. A randomized controlled trial in sheep. Am J Respir Crit Care Med 2020;202:383-92.
13. Millar JE, Wildi K, Bartnikowski N, et al. Characterizing preclinical sub-phenotypic models of acute respiratory distress syndrome: an experimental ovine study. Physiol Rep 2021;9:e15048.
14. Bouquet M, Passmore MR, See Hoe LE, et al. Development and validation of ELISAs for the quantitation of interleukin (IL)-1beta, IL-6, IL-8 and IL-10 in ovine plasma. J Immunol Methods 2020:112835.
15. Heinsar S, Jung JS, Colombo SM, et al. An innovative ovine model of severe cardiopulmonary failure supported by veno-arterial extracorporeal membrane oxygenation. Sci Rep 2021;11:20458.
16. Byrne L, Obonyo NG, Diab SD, et al. Unintended consequences: fluid resuscitation worsens shock in an ovine model of endotoxemia. Am J Respir Crit Care Med 2018;198:1043-54.
17. Casals M, Girabent-Farres M, Carrasco JL. Methodological quality and reporting of generalized linear mixed models in clinical medicine (2000-2012): a systematic review. PLoS One 2014;9:e112653.
18. Barr DJ, Levy R, Scheepers C, Tily HJ. Random effects structure for confirmatory hypothesis testing: keep it maximal. J Mem Lang 2013: 68.
19. Venema CS, Erasmus ME, Mariani M, Voors AA, Damman K. Post-transplant inotrope score is associated with clinical outcomes after adult heart transplantation. Clin Transplant 2021;35:e14347.
20. Dronavalli VB, Rogers CA, Banner NR. Primary cardiac allograft dysfunction-validation of a clinical definition. Transplantation 2015;99:1919-25.
21. Sanil Y, Aggarwal S. Vasoactive-inotropic score after pediatric heart transplant: a marker of adverse outcome. Pediatr Transplant 2013; 17:567-72.
22. Kedziora A, Piatek J, Hymczak H, et al. Early postoperative hemodynamic instability after heart transplantation - incidence and metabolic indicators. BMC Anesthesiol 2021;21:236.
23. Lim HS, Ranasinghe A, Quinn D, Chue CD, Mascaro J. Pathophysiology of severe primary graft dysfunction in orthotopic heart transplantation. Clin Transplant 2021;35:e14398.
24. Kedziora A, Wierzbicki K, Piatek J, et al. Postoperative serum lactate levels for in-hospital mortality prediction among heart transplant recipients. Ann Transplant 2020;25:e920288.
25. Anderson NM, Bond GY, Joffe AR, et al. Post-operative fluid overload as a predictor of hospital and long-term outcomes in a pediatric heart transplant population. Pediatr Transplant 2021;25:e13897.
26. Iyer A, Kumarasinghe G, Hicks M, et al. Primary graft failure after heart transplantation. J Transplant 2011;2011:175768.
27. Foroutan F, Alba AC, Stein M, et al. Validation of the International Society for Heart and Lung Transplantation primary graft dysfunction instrument in heart transplantation. J Heart Lung Transplant 2019;38:260-6.
28. See Hoe LE, Wells MA, Bartnikowski N, et al. Heart transplantation from brain dead donors: a systematic review of animal models. Transplantation 2020;104:2272-89.
29. Novitzky D, Rhodin J, Cooper DK, Ye Y, Min KW, DeBault L. Ultrastructure changes associated with brain death in the human donor heart. Transpl Int 1997;10:24-32.
30. Novitzky D, Rose AG, Cooper DK. Injury of myocardial conduction tissue and coronary artery smooth muscle following brain death in the baboon. Transplantation 1988;45:964-6.
31. Cooper DK, Novitzky D, Wicomb WN. The pathophysiological effects of brain death on potential donor organs, with particular reference to the heart. Ann R Coll Surg Engl 1989;71:261-6.
32. Belhaj A, Dewachter L, Rorive S, et al. Roles of inflammation and apoptosis in experimental brain death-induced right ventricular failure. J Heart Lung Transplant 2016;35:1505-18.
33. Michel SG, Muraglia GM La 2nd, Madariaga ML, et al. Preservation of donor hearts using hypothermic oxygenated perfusion. Ann Transplant 2014;19:409-16.
34. Michel SG, Muraglia GM La 2nd, Madariaga ML, et al. Twelve-hour hypothermic machine perfusion for donor heart preservation leads to improved ultrastructural characteristics compared to conventional cold storage. Ann Transplant 2015;20:461-8.
35. Cobert ML, West LM, Jessen ME. Machine perfusion for cardiac allograft preservation. Curr Opin Organ Transplant 2008;13:526-30.

36. Tatum R, O'Malley TJ, Bodzin AS, Tchantchaleishvili V. Machine perfusion of donor organs for transplantation. Artif Organs 2021;45:682-95.
37. Steen S, Sjoberg T, Liao Q, Bozovic G, Wohlfart B. Pharmacological normalization of circulation after acute brain death. Acta Anaesthesiol Scand 2012;56:1006-12.
38. Fuller BJ, Lee CY. Hypothermic perfusion preservation: the future of organ preservation revisited? Cryobiology 2007;54:129-45.
39. Tago M, Seno S, Bando K, Teraoka H, Senoo Y, Teramoto S. [High energy phosphates and mitochondrial respiratory function after 24 hours cardiac preservation—comparison between simple hypothermic immersion and continuous hypothermic perfusion]. Kokyu To Junkan 1989;37:61-3.
40. Qin G, Wohlfart B, Zuo L, Hu J, Sjoberg T, Steen S. Intact coronary and myocardial functions after 24 hours of non-ischemic heart preservation. Scand Cardiovasc J 2020;54:59-65.
41. Miller CL, Madsen JC. IL-6 directed therapy in transplantation. Curr Transplant Rep 2021: 1-14.
42. Liang Y, Christopher K, Finn PW, Colson YL, Perkins DL. Graft produced interleukin-6 functions as a danger signal and promotes rejection after transplantation. Transplantation 2007;84:771-7.
43. Zhao X, Boenisch O, Yeung M, et al. Critical role of proinflammatory cytokine IL-6 in allograft rejection and tolerance. Am J Transplant 2012;12:90-101.
44. Booth AJ, Grabauskiene S, Wood SC, Lu G, Burrell BE, Bishop DK. IL-6 promotes cardiac graft rejection mediated by CD4+ cells. J Immunol 2011;187:5764-71.
45. Schroder J, D'Alessandro D, Esmailian F, et al. Successful utilization of Extended Criteria Donor (ECD) hearts for transplantation - results of the OCS™ Heart EXPAND Trial to evaluate the effectiveness and safety of the OCS heart system to preserve and assess ECD hearts for transplantation. J Heart Lung Transplant 2019;38: S42.
46. Messer S, Page A, Colah S, et al. Human heart transplantation from donation after circulatory-determined death donors using normothermic regional perfusion and cold storage. J Heart Lung Transplant 2018;37:865-9.
47. Avtaar Singh SS, Banner NR, Rushton S, Simon AR, Berry C, Al-Attar N. ISHLT primary graft dysfunction incidence, risk factors, and outcome: A UK National Study. Transplantation 2019;103:336-43.
48. De Blasi RA, Palmisani S, Cigognetti L, et al. Effects of sternotomy on heart-lung interaction in patients undergoing cardiac surgery receiving pressure-controlled mechanical ventilation. Acta Anaesthesiol Scand 2007;51:441-6.
49. Sander M, Spies CD, Berger K, et al. Prediction of volume response under open-chest conditions during coronary artery bypass surgery. Crit Care 2007;11:R121.