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Purpose: The key goal of cold storage is to maintain cell viability for a prolonged time during solid organ transplantation. Methane (CH_4) has been recognized as novel therapeutic gas exerting anti-inflammatory effects in ischemia-reperfusion (IR) injuries. We aimed to investigate whether cold storage of donor hearts in CH_4-enriched Custodiol preservation solution could protect against IR and preserve myocardial function in a rat model of heterotopic heart transplantation (HTX).
Methods: The hearts of donor Lewis rats were explanted and stored for 1 h in cold Custodiol (CS group, n=12) or in CH_4-saturated (0.054 mg/100 ml) Custodiol (CS-CH_4 group, n=12). 60 min after HTX left ventricular (LV) pressure-volume relations and coronary blood flow (CBF) were assessed to evaluate early post-transplant graft function. At the end of haemodynamic measurements, samples were taken for qPCR of endoplasmic reticulum (ER) stress and mitochondria-related apoptosis markers (CHOP, GRP78, GSK3β, VLDR, Caspase 3 and 9, Bcl2, Bax), biochemical parameters and mitochondrial functional analysis with high-resolution respirometry (Oxygraph2K, Austria).
Results: LV contractility (LV systolic pressure at 120μl of LV volume: 86±6 vs. 57±7mmHg, p=0.01; dP/dt_{max}: 2326±167 vs. 1583±139mmHg, p=0.007) and active relaxation (dP/dt_{min} at 120μl of LV volume: -1660±185 vs. -1043±169mmHg, p=0.04) improved significantly after an hour of reperfusion, while alteration of CBF standardized to heart weight (2.11±0.35 vs. 1.13±0.2ml/min/g, p=0.04) was also significantly improved following pretreatment. CS-CH_4 storage significantly reduced the transcription of pro-apoptotic proteins and Bcl2/Bax ratios as compared to CS grafts. Increased mitochondrial oxidative phosphorylation, reduced leak respiration and cytochrome c release were demonstrated in response to CS-CH_4 preservation.
Conclusion: These results might provide reliable evidence for the benefit of CH_4-enriched preservation solution during HTX, through a mechanism which involves the inhibition of pro-apoptotic signals. Hence CH_4-enriched preservation solution could be a potential cardioprotective agent in the inventory of heart transplantation surgery and other cardiac surgical procedures requiring prolonged cardioplegia.

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Assessment of Cerebral Perfusion and Activity during Normothermic Regional Perfusion in a Porcine Model of Donation after Circulatory Death

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Purpose: To determine cerebral perfusion/activity during normothermic regional perfusion (NRP) in a model of donation after circulatory death (DCD). NRP is a resuscitation strategy used for DCD hearts involving *in situ* reperfusion of the donor excluding cerebral circulation. Ethical concerns have been raised regarding potential return of spontaneous cerebral activity due to collateral circulation
Methods: We induced circulatory arrest by stopping mechanical ventilation in 6 donor pigs. A 15-minute stand-off period followed before resuscitation with NRP. We performed NRP via central cannulation with the supra-aortic vessels clamped and anesthetic drugs suspended. We assessed cerebral activity using Bispectral Index (BIS) and tested the presence of brainstem reflexes (pupillaryresponse, oculocephalic and corneal reflex, and spontaneous respirations). Cerebral perfusion was assessed by continuous oximetry with Near-Infrared Spectroscopy (NIRS) and a cerebral angiography
Results: Brainstem reflexes were uniformly absent at all time points following DCD in all cases. NIRS decreased markedly during DCD induction and became absent during NRP. A slight increase in oxygenation was noted in Case 1 during NRP; however, this stabilized at non-viable oxygenation levels. This case also demonstrated flow through collateral circulation via internal mammary arteries on angiography, but no cerebral activity was detected. BIS monitoring demonstrated absence of cerebral activity during NRP
Conclusion: Our findings suggest that, although occasional collateral brain flow may occur, there is no significant brain perfusion or return of function during NRP. NRP is safe to be performed clinically and can enhance DCD donor organ utilization, especially in heart transplantation. Future studies are required focusing on the impact of NRP over DCD organ functional recovery and clinical outcomes

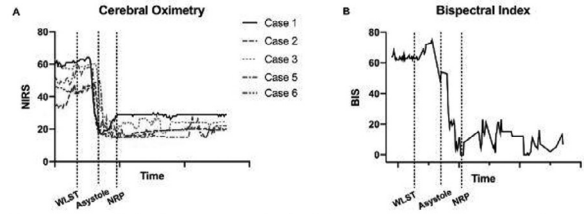

Figure 1. A: Cerebral oximetry measured using Near-infrared spectroscopy demonstrates an acute decrease in cerebral oxygenation during DCD induction and NRP. B: Representative Bispectral index measurements from Case 4. BIS also demonstrates an acute decline during DCD and remains absent during NRP. Some artifacts are noticed as spikes in measurements.

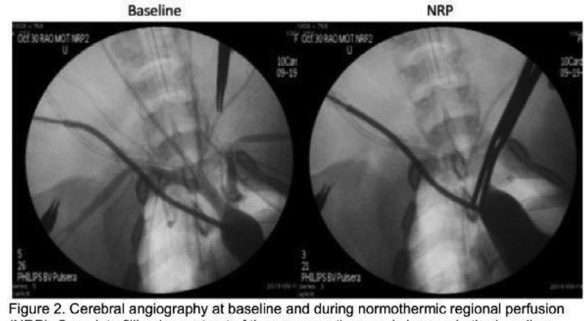

Figure 2. Cerebral angiography at baseline and during normothermic regional perfusion (NRP). Complete filling by contrast of the supra-aortic vessels is seen in the baseline image. During NRP, a cross-clamp is placed over the supra-aortic vessels to exclude cerebral circulation and no contrast is seen.

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Comparison of Continuous Hypothermic Oxygenated Crystalloid Perfusion with the Novel Solution "Custodiol N " and Warm Blood Perfusion in a Porcine DCD Donation Model

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Purpose: Transplantation programs are worldwide burdened by the limited availability of suitable donor hearts, despite extending the donation criteria. Organ donation after circulatory death (DCD) became a reliable alternative over the past few years. Nevertheless, the grafts fetched as DCDs are submitted to a further ischemic insult due to the progressive hypoxia and circulatory arrest occurring between withdrawal of care and declaration of death. Although diverse storage and perfusion strategies exist to preserve DCD hearts, no direct comparison has yet been performed. The aim of the present study is to examine the effects of a novel cold crystalloid preservation solution (Custodiol-N) and warm blood perfusion protocols compared to simple cold storage in a porcine model.
Methods: In 39 pigs, DCD was induced by asphyxia and the hearts underwent 30 min initial warm ischemia in the donor. The hearts were than explanted and divided in 4 groups: 1) control group (n=8) was placed directly in a Langendorff model. In group 2 (CSG, n=8) the hearts were flushed with 2L of 4˚C Custodiol N solution and then stored in the same solution for 4h before evaluation. In group 3 (CCP, n=8), the hearts underwent a 4h continuous oxygenated 4˚C cold machine perfusion cycle with Custodiol N. In group 4 (WBP, n=7) the hearts were placed on warm blood reperfusion for 4h. Left ventricular function was defined by dP/dt min & max at different ventricle filling volumes. Coronary blood flow (CBF) was measured at 100 mmHg perfusion pressure and Troponin T was collected up to 2 hours.

Results: Both dP/dt min & max were significantly higher in the CPP group compared to the WBP group [-1038±182 vs -344±38 mmHg/s; p=0.0356 and 1441±194 vs 498±53 mmHg/s; p=0.0123 respectively]. CBF was also significantly higher in the CCP group [1145±57vs 726±72ml/min; p=0.0032]. Moreover, Troponin T was significantly elevated in the WBP compared to the CCP group [27477±11777 vs 251034±90247 pg/ml, p=0.0239]. There were no significant differences among the WBP, CSG and control groups.
Conclusion: Continuous hypothermic oxygenated crystalloid perfusion with the novel preservation solution Custodiol N offers superior preservation and resuscitation of DCD hearts in terms of functional recovery and myocardial enzyme release.

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Successful Transplantation of Porcine Lungs Following 3 Days of Preservation Using a Modified Cold Static Method Paired with Intermittent Normothermic Ex Vivo Lung Perfusion (EVLP)

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Purpose: We have recently showed the superiority of 10˚C cold static preservation (CSP) compared to standard 4˚C. Despite that, CSP cannot be prolonged unlimited. Here, we hypothesized that combining CSP at 10˚C with 2 short cycles of normothermic EVLP (a "recharge" period) would further extend donor lung preservation.
Methods: Donor lungs (n=3) from Yorkshire pigs (28-35kg) were flushed with 4˚C LPD and subsequently preserved using 10˚C CSP and intermittent normothermic EVLP (Fig 1A). After a total of 72h of preservation, a left lung transplant was performed followed by 4h of reperfusion. At 4h of reperfusion, isolated graft assessment was performed by clamping the contralateral pulmonary artery and bronchus. To evaluate the contribution of the EVLP recharge periods, 2 control lungs were preserved solely with 10˚C CSP for 72h and assessed using normothermic EVLP.
Results: After 3-days of preservation, post-transplant graft function was excellent. Systemic P/F ratio after excluding the contra-lateral lung was 430 ± 57 mmHg. Lung function was also stable during the intermittent EVLP periods (Fig 1B) and after transplantation (Fig 1C). No pulmonary edema was observed in the bronchoscopic assessment after transplant. Lungs preserved purely in CSP for 72h were assessed on EVLP and failed immediately with the development of massive pulmonary edema.
Conclusion: We demonstrate for the first time the feasibility of 3-day lung preservation leading to excellent early post-transplant outcomes. Further experiments are being performed to confirm these positive findings and underlying mechanisms. The combination of a modified CSP method (10˚C) and intermittent EVLP can open new opportunities to further prolong organ preservation and provide time for advanced lung treatments and repair.

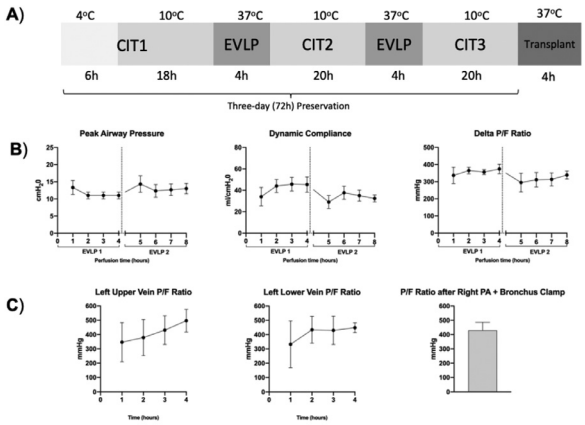

Fig 1. Experimental Design and Physiologic results during EVLP and Post-transplant phase. (A) Donor lungs were stored at 4°C for 6h of cold ischemic time (CIT) to simulate transportation. Lungs were then stored at 10°C for 18h, which was then followed by normothermic ex vivo lung perfusion (EVLP) for 4h. After EVLP, lungs underwent static storage at 10°C for another 20h, and then placed on EVLP again for 4h. Lungs were stored again for 20h at 10°C, transplanted into a recipient pig, and reperfused for 4h. (B) Physiologic assessments during two EVLP recharge periods. (C) Functional analysis of transplanted graft oxygenation during reperfusion. All results are expressed as mean ± standard error of measurement.

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Protective Effects of Necrosulfonamide on Ischemia-Reperfusion Injury in Rat Lung

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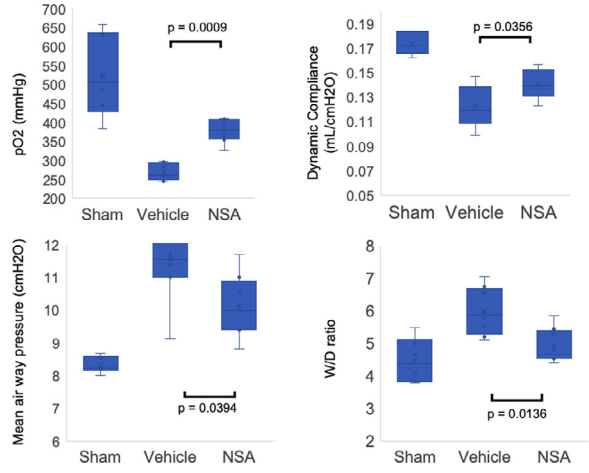
Purpose: In lung transplantation, ischemia-reperfusion injury (IRI) plays a critical role in the development of primary graft dysfunction. Among cell deaths induced by IRI, regulated necrosis has been garnering much attention. Above all, necroptosis is well known, and there are some reports on necroptosis inhibition attenuated IRI improvement in solid organ transplantation. However, there are few reports about mixed lineage kinase domain-like protein (MLKL), which is proposed as a crucial mediator of necroptosis. The purpose of this study was to verify the hypothesis that the MLKL inhibitor necrosulfonamide, (NSA) attenuates IRI.
Methods: Male Lewis rats were heparinized and subjected to left thoracotomy under the anesthetization. Then, the left hilum was clamped for 90 min, followed by reperfusion for 120 min (rat hilar clamp model). NSA 0.5 mg and the solvent were intraperitoneally administered 30 min before ischemia in the NSA group and the vehicle group, respectively (n = 8, for both). After reperfusion, arterial blood gas analysis, physiological data (dynamic compliance, mean, and peak airway pressure), lung wet-to-dry weight ratio (W/D), and histological findings (extravascular red blood cell and neutrophil count and vascular edema) were evaluated under the right hilum occlusion.
Results: The NSA group had higher arterial oxygenation than the vehicle group (p = 0.0009). Dynamic compliance was higher and peak and mean airway pressures were lower in the NSA group than in the vehicle group (p = 0.0356, p = 0.0181, and p = 0.0394, respectively). W/D ratio was also significantly improved in NSA group (p = 0.0136). Histologically, hemorrhage and neutrophil in alveolar or interstitial space were significantly lower in the NSA group (p = 0.0357, p = 0.0157, respectively). Perivascular edema evaluated by vascular cuff was also ameliorated in the NSA group as well W/D ratio (p = 0.000155).
Conclusion: Our results suggested that NSA alleviated lung IRI via necroptosis inhibition in rat lung.



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Normothermic Regional Perfusion (NRP) during Heart DCD Recovery: Is Lung Quality Impacted? A Pre-Clinical Study

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