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Experimental paper

A randomized and blinded trial of inhaled nitric oxide in a piglet model of pediatric cardiopulmonary resuscitation

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Abstract

Aim: Inhaled nitric oxide (iNO) during cardiopulmonary resuscitation (CPR) improved systemic hemodynamics and outcomes in a preclinical model of adult in-hospital cardiac arrest (IHCA) and may also have a neuroprotective role following cardiac arrest. The primary objectives of this study were to determine if iNO during CPR would improve cerebral hemodynamics and mitochondrial function in a pediatric model of lipopolysaccharide-induced shock-associated IHCA.

Methods: After lipopolysaccharide infusion and ventricular fibrillation induction, 20 1-month-old piglets received hemodynamic-directed CPR and were randomized to blinded treatment with or without iNO (80 ppm) during and after CPR. Defibrillation attempts began at 10 min with a 20-min maximum CPR duration. Cerebral tissue from animals surviving 1-h post-arrest underwent high-resolution respirometry to evaluate the mitochondrial electron transport system and immunohistochemical analyses to assess neuropathology.

Results: During CPR, the iNO group had higher mean aortic pressure (41.6 ± 2.0 vs. 36.0 ± 1.4 mmHg; p = 0.005); diastolic BP (32.4 ± 2.4 vs. 27.1 ± 1.7 mmHg; p = 0.03); cerebral perfusion pressure (25.0 ± 2.6 vs. 19.1 ± 1.8 mmHg; p = 0.02); and cerebral blood flow relative to baseline (rCBF: 243.2 ± 54.1 vs. 115.5 ± 37.2%; p = 0.02). Among the 8/10 survivors in each group, the iNO group had higher mitochondrial Complex I oxidative phosphorylation in the cerebral cortex (3.60 [3.56, 3.99] vs. 3.23 [2.44, 3.46] pmol O_2/s mg; p = 0.01) and hippocampus (4.79 [4.35, 5.18] vs. 3.17 [2.75, 4.58] pmol O_2/s mg; p = 0.02). There were no other differences in mitochondrial respiration or brain injury between groups.

Conclusions: Treatment with iNO during CPR resulted in superior systemic hemodynamics, rCBF, and cerebral mitochondrial Complex I respiration in this pediatric cardiac arrest model.

Keywords: In-hospital cardiac arrest, Cardiopulmonary resuscitation, Pediatrics, Shock, Pulmonary hypertension, Inhaled nitric oxide, Physiology, Cerebral blood flow, Hemodynamics, Laboratory

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Introduction

Thousand of children have in-hospital cardiac arrests (IHCAs) annually[1] and while previously improving, survival rates from pediatric IHCA have plateaued in recent years. More than half of children with IHCA do not survive to hospital discharge[2,3] and among survivors, neurologic morbidity is common.[4] Although high-quality cardiopulmonary resuscitation (CPR) and epinephrine are associated with improved outcomes from pediatric IHCA, identification and evaluation of other therapeutics is necessary to improve patient outcomes.[5,6]

Inhaled nitric oxide (iNO) is used frequently as a pulmonary vasodilator in critically ill children with pulmonary hypertension (PH) or refractory hypoxemic respiratory failure.[7] Additionally, iNO and intravenous nitrites may offer post-arrest neuroprotection through reduction of mitochondrial reactive oxygen species (mtROS) generation, protein S-nitrosation to promote pro-survival neuronal signaling pathways, and other potential protective mechanisms.[8–11] In a previous laboratory investigation, our group demonstrated superior intra-arrest hemodynamics and improved short-term survival when iNO was administered during CPR in a swine model of shock- and PH-associated adult IHCA.[12] These findings suggested that iNO lowered pulmonary vascular resistance (PVR) and improved blood flow during CPR, but the study did not evaluate cerebral hemodynamics or neurologic injury.

In the present study, we sought to extend previous work to a pediatric model, as shock and PH are frequently present at the time of arrest in hospitalized children.[13,14] We also aimed to evaluate intra- and post-arrest markers of neurologic function and injury. We hypothesized that randomized and blinded administration of iNO during CPR in a porcine model of lipopolysaccharide-induced shock- and PH-associated pediatric IHCA would result in: 1) higher systemic blood pressures and cerebral blood flow (CBF) during CPR; 2) improved mitochondrial bioenergetics; 3) decreased generation of mtROS in the brain following CPR; and 4) decreased neuropathologic injury as measured by oxidative injury, apoptosis, and inflammation markers in the brain following CPR.

Methods

Animal preparation and data collection

Experimental procedures were in concordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC). Full methodological details are available in previous publications.[12,15–18] Briefly, 20 one-month-old domestic swine (*Sus scrofa domesticus*; n = 10 of each sex) were randomized, anesthetized, and mechanically ventilated. Vascular catheters were placed in the right atrium, and aorta for continuous hemodynamic measurements. A Swan-Ganz catheter was placed in the pulmonary artery (PA) for hemodynamic measurements, arterial blood gas acquisition, and intermittent cardiac output measurement via thermodilution. Through burr holes in the right frontoparietal skull, a brain tissue oxygenation probe (Licox, Integra LifeSciences, Plainsboro, New Jersey, USA) was placed 1 cm into brain tissue and a laser Doppler probe (Periflux, Perimed AB, Sweden) was positioned overlying the dura mater to measure CBF. The electrocardiogram, aortic pressure, right atrial (RA) pressure, PA pressure, coronary perfusion pressure (CoPP), pulse oximetry, end-tidal carbon dioxide ($ETCO_2$), laser Doppler-determined relative CBF (rCBF), and brain tissue oxygen tension ($PbtO_2$) were recorded in PowerLab (ADInstruments, Colorado Springs, Colorado, USA) at a sampling rate of 100 −1000 Hz. A CPR quality-recording defibrillator (Zoll R Series; Zoll Medical Corporation, Chelmsford, Massachusetts, USA) measured chest compression rate (compressions/minute) and depth (cm). Arterial and venous blood samples were acquired at predetermined times throughout the experiment for blood gas analyses and plasma was stored for additional analyses.

Resuscitation protocol

Lipopolysaccharide (LPS; *Escherichia coli* 055:B5; Sigma, St. Louis, Missouri, USA) was administered via intravenous infusion to induce a systemic inflammatory response and pulmonary hypertension. The initial rate of the infusion was 7 mcg/kg/h and was increased stepwise (7, 14, and 20 mcg/kg/h) every 10 min to a maximum rate of 20 mcg/kg/h based on previous studies.[12,19] After 45 total minutes, LPS was discontinued and ventricular fibrillation (VF) was electrically induced via transthoracic pacing needles in the right ventricle. If the animal developed hypotension with mean arterial pressure (MAP) <40 mmHg prior to 45 min, LPS was stopped and VF was induced at that time. Induction of VF facilitated a consistent minimum duration of CPR in which to study the interventions.[12,15,17]

Upon confirmation of VF, manual CPR commenced for 10 min prior to an initial defibrillation attempt. Compressions were delivered at a rate of 100 per minute with guidance from a metronome and the CPR quality-monitoring defibrillator. Mechanical ventilations (tidal volume 10 mL/kg, positive end-expiratory pressure 5 cm H_2O, rate 10 per minute with 100% inhaled oxygen) were provided throughout CPR. All animals received HD-CPR as previously detailed[12,15–18,20] with chest compression force actively titrated to an aortic systolic pressure of 90 mmHg[21] and vasopressors (epinephrine and vasopressin) administered to maintain CoPP ≥20 mmHg.

Animals were randomized to treatment with or without iNO. The INOmax delivery system (Mallinckrodt Pharmaceuticals, Dublin, Ireland) was in-line with the ventilator in all experiments with the display screen concealed such that a sole unblinded monitor knew if iNO was provided. Beginning one minute into CPR, that individual provided either iNO (80 ppm) or no iNO and was not otherwise involved in the study. All other investigators remained blinded to group assignment until analyses were complete.

Ten minutes of CPR was provided to all animals followed by an initial defibrillation attempt (50 Joules). In animals without immediate ROSC, resuscitation continued for up to ten additional minutes with defibrillation attempts every two minutes as indicated by rhythm. Animals with ROSC received standardized post-arrest care including intravenous crystalloid and epinephrine (infusion and bolus dosing) to meet MAP goals (45−60 mmHg). In the iNO group, animals received iNO until the study's endpoint.

Euthanasia, tissue acquisition, and tissue analyses

One-hour post-ROSC, a craniotomy was performed to expose the brain. Animals were humanely euthanized under anesthesia with potassium chloride, with simultaneous brain tissue extraction. Left frontal cortex and hippocampus were rapidly excised and placed in ice-cold buffer. Fresh tissue underwent immediate mitochondrial respirometry and additional tissue was frozen for neuropathology and cytokine assays.

Assessment of mitochondrial respiration in the cortex and hippocampus was performed via high-resolution respirometry (Oxygraph-2K; OROBOROS Instruments, Innsbruck, Austria). A substrate-uncoupler-inhibitor titration (SUIT) protocol was utilized to measure complex-specific oxidative phosphorylation, maximal oxidative phosphorylation, and inner membrane leak respiration.[22–24] Oxygen consumption was recorded with DatLab software (OROBOROS Instruments) and normalized according to citrate synthase as a measure of mitochondrial content. For mtROS quantification, superoxide dismutase was used to transform superoxide, the most proximal ROS product of mitochondrial respiration, to hydrogen peroxide (H_2O_2), which was detected using an Amplex UltraRed assay (Invitrogen, Carlsbad, California, USA) and the Oxygraph-2K high-resolution respirometer equipped with optical sensors (O2K-Fluo LED2-Module, OROBOROS Instruments).

Frozen cortical tissue underwent pathological assessment for quantification of neuronal apoptosis and microglial activation by a blinded nueropathologist. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining (ApopTag Plus In Situ Apoptosis Detection Kit; Millipore Sigma, Burlington, Massachusetts, USA) was performed according to the manufacturer's protocols. The number of apoptotic cells per low-powered (40×) field was quantified using a BX40 microscope (Olympus Corp., Tokyo, Japan). Evaluation of microglial activation was performed with immunohistochemical quantification of ionized calcium binding adaptor molecule-1 (IBA-1) expression. An anti-IBA-1 rabbit polyclonal antibody (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) was applied, followed by Harris hematoxylin (Leica Biosystems, Buffalo Grove, Illinois, USA) staining and semi-quantitative ranking from 1 to 3 (least to most expression).

Enzyme-linked immunosorbent assays (ELISA) with a commercially available kit (Q-Plex porcine cytokine – high sensitivity 4-plex; Quansys Biosciences, Logan, Utah, USA) were performed on ROSC to measure interleukin-1β (IL-1β), IL-6, IL-8, and tumor necrosis factor-α (TNFα). Sample solutions were prepared using a mixture of protease inhibitor and plasma/brain homogenate (1:10 ratio) and sample diluents were added to each sample per manufacter protocol. The plate was imaged using Quansys Imager LS (Quansys Biosciences).

Statistical analyses

Outcomes included: 1) rCBF, CePP, and $PbtO_2$; 2) systemic and pulmonary hemodynamics; 3) chest compression mechanics; 4) ROSC and 1-h post-arrest survival; 5) arterial and venous blood gas measurements; 6) cerebral cortical and hippocampal mitochondrial respiration; 7) histopathologic findings; and 8) peripheral blood and cerebral cytokines.

Survival outcomes were compared between groups using Fisher's exact test. Continuous physiologic data were evaluated as 15-s epochs within each period of the experimental protocol. Values at predetermined timepoints were assessed for normality with the Skewness–Kurtosis test. Normally distributed continuous variables were described as means with standard errors of the mean and evaluated with Student's *t*-test. Non-normally distributed continuous variables were described as medians with interquartile ranges and evaluated with the Wilcoxon rank-sum test. Physiologic variables during the full duration of the LPS period, the CPR period (minutes 1 –10), and the post-resuscitation period were compared with a generalized estimating equation regression model that accounted for clustering of epochs within animals. To assess the physiologic effects of LPS, baseline values were compared to values in the final five minutes of the LPS infusion using a paired *t*-test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. Intra-arrest rCBF values were normalized to the first

Table 1 – Physiologic measurements during LPS infusion.

	HD-CPR with iNO (n = 10)	HD-CPR (n = 10)	*p*
Heart rate (beats per minute)	120.9 (12.7)	119.0 (9.0)	0.88
Oxygen saturation (%)	95.2 (0.7)	95.3 (0.5)	0.86
Mean aortic pressure (mmHg)	73.2 (4.6)	71.1 (3.3)	0.64
Mean right atrial pressure (mmHg)	11.1 (3.0)	10.6 (2.2)	0.88
Mean pulmonary artery pressure (mmHg)	31.2 (5.5)	28.3 (3.9)	0.60
Coronary perfusion pressure (mmHg)	55.6 (5.5)	54.3 (3.89)	0.81
Aortic diastolic pressure (mmHg)	66.8 (4.9)	64.9 (3.4)	0.70
Aortic systolic pressure (mmHg)	84.1 (4.9)	81.8 (3.5)	0.64
Cardiac output – 30 min (L/min)[a]	0.80 (0.08)	0.67 (0.09)	0.30
End-tidal carbon dioxide (mmHg)	38.6 (1.1)	38.0 (0.8)	0.57
Brain tissue oxygenation (mmHg)	14.5 (3.4)	13.3 (2.4)	0.71
Relative cerebral blood flow (% baseline)[b]	108.6 (10.8)	96.7 (7.4)	0.27
Cerebral perfusion pressure (mmHg)[c]	62.1 (5.3)	60.4 (3.8)	0.75
Duration of LPS infusion (min)[d]	36.5 (4.3)	40.0 (3.3)	0.53

Definition of abbreviations: HD-CPR = hemodynamic-directed cardiopulmonary resuscitation; iNO = inhaled nitric oxide.
Unless otherwise noted, values represent mean from LPS infusion period (45 min in animals receiving full duration of LPS infusion), compared using generalized estimating equation regression model. Parenthetic numbers indicate SEM.
[a] For intermittent measurements, data distribution for each variable determined with Skewness–Kurtosis test. All data were normally distributed and are displayed as mean and standard error of the mean (in parentheses) and compared using Student's *t*-test.
[b] Calculated as the percent of the pre-LPS infusion baseline value.
[c] Calculated as difference between MAP and RAP.
[d] Three animals in HD-CPR with iNO group and two animals in HD-CPR group met criteria for VF induction prior to 45 min and thereby received <45 min of LPS infusion.

cerebral and hippocampal tissue as well as plasma acquired 1-h post-

minute of CPR (prior to iNO initiation) due to the arbitrary nature of the raw data output (perfusion units).

7Mitochondrial respiration measurements, mtROS values, and cytokine values were compared between groups using the Wilcoxon rank-sum test. The Bonferroni correction was applied to cytokine values to account for repeated measurements within each tissue. Histopathology findings were compared with the chi-square test. Statistical analyses were performed with the Stata IC statistical package (Version 14; StataCorp, College Station, Texas, USA) and GraphPad Prism (Version 8; GraphPad, San Diego, California, USA). P-values <0.05 were considered significant (<0.0125 for cytokine values).

Results

The iNO group had lower cardiac output at baseline. There were no other differences between groups at baseline (Supplemental Table 1) or during the LPS infusion (Table 1). Relative to baseline values, at the conclusion of the LPS infusion, animals had higher mean PA pressures (28.1 ± 2.8 mmHg vs. 15.3 ± 0.9 mmHg; $p < 0.001$), lower oxygen saturation (95.9 [94.0, 97.2]% vs. 97.7 [95.3, 98.1]%; $p = 0.02$), lower pH (7.37 ± 0.02 vs. 7.46 ± 0.02; $p < 0.001$), and higher arterial lactate (1.5 ± 0.2 mmol/L vs. 1.2 ± 0.1 mmol/L; $p = 0.02$). Other physiologic measurements did not change significantly from baseline values (Supplemental Table 2). Two animals in the control group and three animals in the iNO group met criteria for termination of LPS and induction of VF prior to 45 min. The median duration of the LPS infusion did not differ between groups.

During CPR, the iNO group had higher MAP (41.6 ± 2.0 mmHg vs. 36.0 ± 1.4 mmHg; $p = 0.005$), higher DBP (32.4 ± 2.4 mmHg vs. 27.1 ± 1.7; $p = 0.03$), higher CePP (25.0 ± 2.6 mmHg vs. 19.1 ± 1.8 mmHg; $p = 0.02$), and higher rCBF (percent of baseline CPR value) (243.2 ± 54.1% vs. 115.5 ± 37.3%; $p = 0.02$) (Table 2; Fig. 1). The iNO group had higher mixed venous oxygen saturation seven minutes into CPR (21.5 [15.0, 42.0]% vs. 12.0 [10.0, 14.0]%; $p = 0.045$). There were no other blood gas differences between groups at any timepoint (Supplemental Table 3).

Eight animals in each group (80%) achieved ROSC and survived to the 1-h post-arrest endpoint. Post-arrest, the iNO group continued to have higher CBF relative to pre-experimental baseline (103.9 ± 14.8% vs. 59.5 ± 10.1%; $p = 0.003$). There were no other physiologic differences between groups post-arrest (Table 3).

Animals treated with iNO had higher Complex I-driven oxidative phosphorylation in both the cortex (3.60 [3.56, 3.99] pmol O_2/s mg vs. 3.23 [2.44, 3.46] pmol O_2/s mg; $p = 0.01$) and the hippocampus (4.79 [4.35, 5.18] vs. 3.17 [2.75, 4.58]; $p = 0.02$). There were no other differences in mitochondrial respiration or mtROS (Fig. 2). There were no differences in histopathologic markers of apoptosis or microglial activation or in cytokine concentrations in the brain or plasma (Table 4).

Discussion

In this randomized and blinded preclinical trial in a model of pediatric cardiac arrest associated with LPS-induced systemic inflammation and pulmonary hypertension, the provision of iNO resulted in higher DBP, MAP, and CePP during CPR and higher rCBF both during and following CPR. The iNO group also demonstrated improved Complex I mitochondrial respiration in the brain. These findings are consistent with previous studies demonstrating beneficial effects of iNO during CPR on systemic hemodynamics and neurologic parameters.[9–12,25,26] The novel observation of higher intra-arrest rCBF with iNO may be a mechanistic explanation for enhanced mitochondrial respiration. Cumulatively, these data suggest a potential role for iNO as an intra-arrest therapy and as a focus of prospective clinical investigations.

Table 2 – Physiologic measurements during cardiopulmonary resuscitation.

	HD-CPR with iNO (n = 10)	HD-CPR (n = 10)	*p*
Mean aortic pressure (mmHg)	41.6 (2.00)	36.0 (1.4)	0.005
Mean right atrial pressure (mmHg)	16.6 (1.1)	16.2 (0.8)	0.73
Mean pulmonary artery pressure (mmHg)	29.1 (4.0)	27.3 (2.9)	0.66
Coronary perfusion pressure (mmHg)	20.2 (2.3)	16.2 (1.6)	0.08
Aortic diastolic pressure (mmHg)	32.4 (2.4)	27.1 (1.7)	0.03
Aortic systolic pressure (mmHg)	80.4 (4.8)	84.0 (3.4)	0.45
End-tidal carbon dioxide (mmHg)	24.5 (3.0)	24.0 (2.1)	0.88
Brain tissue oxygenation (mmHg)	28.7 (14.7)	16.7 (10.4)	0.41
Relative cerebral blood flow (% baseline)[a]	243.2 (54.1)	115.5 (37.3)	0.02
Cerebral perfusion pressure (mmHg)[b]	25.0 (2.6)	19.1 (1.8)	0.02
Chest compression rate (min^{-1})	100.8 (0.5)	100.2 (0.3)	0.26
Chest compression depth (cm)	4.2 (0.3)	4.0 (0.2)	0.38
CPR duration (min)	13.2 (1.4)	12.4 (1.3)	0.68
Vasopressor doses (n)	7.8 (1.3)	6.4 (1.2)	0.44
Defibrillation attempts (n)	1 [1,2]	1 [1,2]	0.90

Definition of abbreviations: HD-CPR = hemodynamic-directed cardiopulmonary resuscitation; iNO = inhaled nitric oxide.
Mean values from minutes 1–10 of resuscitation period, compared using generalized estimating equation regression model. Parenthetic numbers indicate SEM. Diastolic pressures and coronary perfusion pressure measured during the release phase of chest compressions. Systolic pressures indicate peak pressures during compression phase.
[a] Calculated as the percent of the CBF value from the first 60 s of CPR.
[b] Calculated as the difference between MAP and RAP.

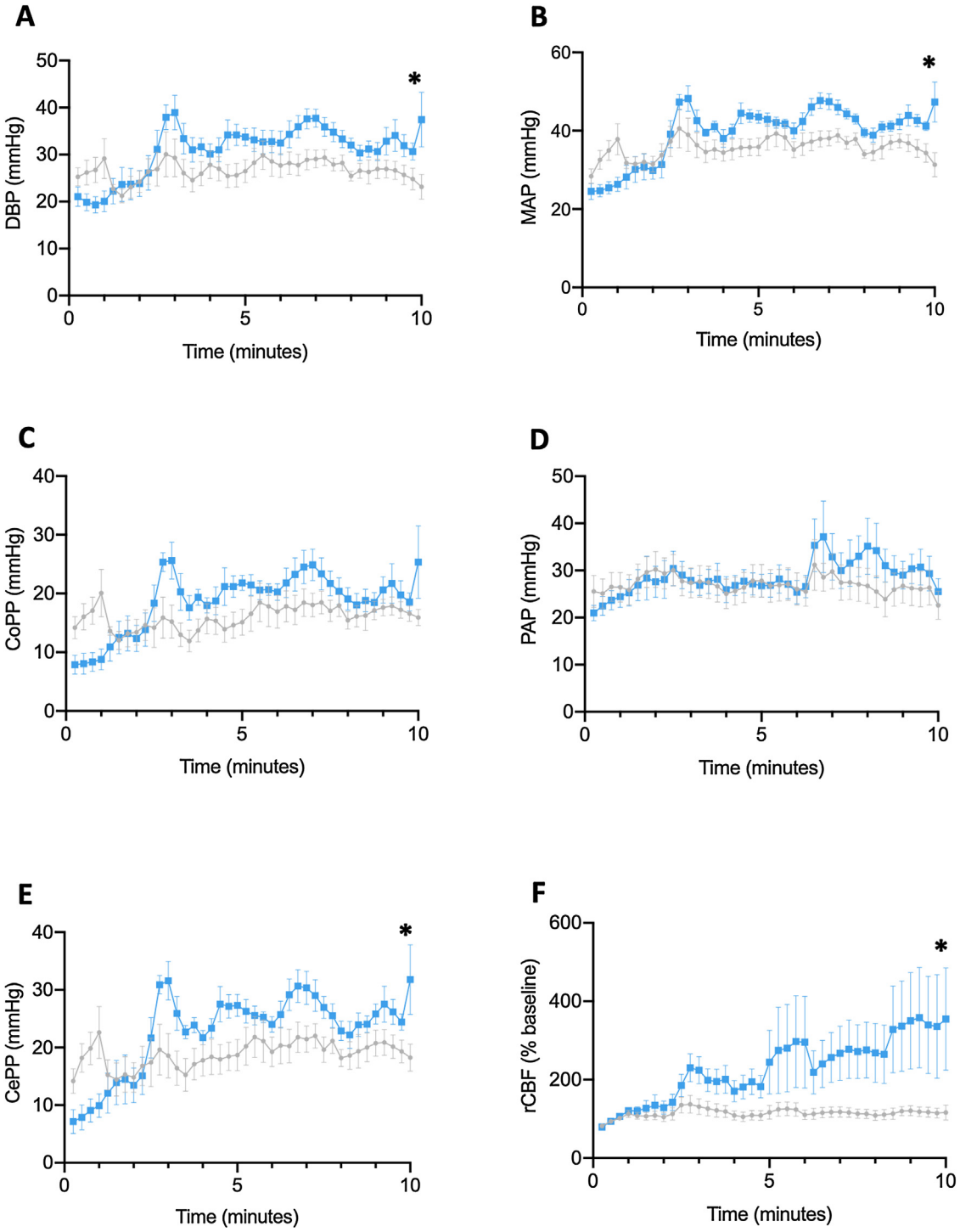

Fig. 1 – Hemodynamics during cardiopulmonary resuscitation.
Graphs represent: A, **aortic diastolic blood pressure (DBP);** B, **mean aortic blood pressure (MAP);** C, **coronary perfusion pressure (CoPP);** D, **mean pulmonary artery pressure (PAP);** E, **cerebral perfusion pressure (CePP); and** F, **relative cerebral blood flow (rCBF) during the first ten minutes of cardiopulmonary resuscitation (CPR). Values are 15-second means for each variable with standard error of the mean depicted with error bars. Blue squares = hemodynamic-directed CPR (HD-CPR) with inhaled nitric oxide (iNO). Gray circles = HD-CPR without iNO. All pressures are mmHg. rCBF is % baseline relative to the first minute (pre-iNO) of CPR. Values from minutes 1–10 compared between groups using a generalized estimating equation accounting for clustering within subjects. *Indicates statistically significant ($p < 0.05$) difference between groups.**

Table 3 – Post-resuscitation physiologic measurements.

	HD-CPR with iNO (n = 10)	HD-CPR (n = 10)	*p*
Heart rate (beats per minute)	117.6 (20.4)	134.1 (14.4)	0.42
Oxygen saturation (%)	95.0 (0.7)	94.9 (0.5)	0.94
Mean aortic pressure (mmHg)	61.2 (2.1)	59.6 (1.5)	0.45
Mean right atrial pressure (mmHg)	7.7 (0.8)	9.1 (0.6)	0.11
Mean pulmonary artery pressure (mmHg)	23.8 (5.0)	20.2 (3.5)	0.46
Coronary perfusion pressure (mmHg)	46.8 (2.8)	43.9 (1.9)	0.29
Aortic diastolic pressure (mmHg)	54.6 (2.9)	53.1 (2.0)	0.60
Aortic systolic pressure (mmHg)	72.2 (3.3)	72.1 (2.3)	0.97
End-tidal carbon dioxide (mmHg)	39.3 (0.9)	38.2 (0.7)	0.24
Cardiac output – 5 min (L/min)[a]	0.54 [0.51, 0.89]	0.63 [0.57, 0.92]	0.49
Cardiac output – 30 min (L/min)[a]	1.10 (0.09)	0.89 (0.14)	0.23
Cardiac output – 55 min (L/min)[a]	1.04 (0.13)	1.00 (0.26)	0.90
Brain tissue oxygenation (mmHg)	16.34 (6.0)	18.2 (4.2)	0.75
Cerebral blood flow (% baseline)[b]	103.9 (14.8)	59.5 (10.1)	0.003
Cerebral perfusion pressure (mmHg)[c]	53.5 (2.3)	50.5 (1.6)	0.19

Definition of abbreviations: HD-CPR = hemodynamic-directed cardiopulmonary resuscitation; iNO = inhaled nitric oxide.
Unless otherwise noted, values represent means from full hour of resuscitation period, compared using generalized estimating equation regression model. Parenthetic numbers indicate SEM.
[a] For intermittent measurements, data distribution for each variable determined with Skewness–Kurtosis test. Normally distributed data displayed as mean and standard error of the mean (in parentheses) and compared using Student's *t*-test. Nonparametric data displayed as median and interquartile range (in brackets) and compared using the Wilcoxon rank-sum test.
[b] Calculated as the percent of the pre-LPS infusion baseline value.
[c] Calculated as difference between MAP and RAP.

Similar to our previous study in an adult swine model[12] and another group's investigation in rats,[25] iNO resulted in higher systemic BPs during CPR. We postulate that this was a result of iNO lowering PVR and increasing pulmonary blood flow,[27] thus generating more pulmonary venous return and enhancing cardiac output during chest compressions. The absence of PA pressure differences between groups is likely due to the countering effects of decreased resistance and increased flow, but we did not directly measure PVR or pulmonary blood flow in this study. End-tidal CO_2, an indirect measurement of pulmonary blood flow, was also not different between groups. While $ETCO_2$ values are an indicator of pulmonary blood flow in the low-flow state of cardiac arrest, they may be less reliable when high-quality CPR is provided and $ETCO_2$ values are relatively high (e.g., >20 mmHg), as they were in this study. In previous work with the HD-CPR strategy used in this study, $ETCO_2$ was also similar between groups despite differences in hemodynamics and survival.[15,17]

The consistent physiologic findings across these investigations offer promise for the role of iNO in improving hemodynamics during IHCA, though further work is necessary to specifically determine which patients may benefit from this intervention. Actual pediatric IHCAs occur in the setting of complex disease processes, and PH during IHCA is seldom solely the result of endotoxemia. Therefore, the degree to which PH during IHCA may be reversed or attenuated by the provision of iNO cannot be determined from this study alone. Further experimental and clinical studies are needed to determine how these findings translate to actual clinical practice. Of note, since the majority of pediatric IHCAs are caused by shock or acute respiratory failure,[14,28] both of which may be accompanied by elevated PVR,[29,30] the role of iNO in pediatric resuscitation may extend beyond just those patients with known PH.

Because of the well-established association of DBP and CoPP during CPR with survival from cardiac arrest,[28,31] we hypothesized that higher BPs would result in higher rates of survival as seen in our previous investigation.[12] However, 80% of animals in the control group survived, likely due to the modest systemic response to LPS and the efficacy of HD-CPR alone in this pediatric model. Further translational and clinical investigations are needed to evaluate iNO's impact on survival.

In addition to higher systemic blood pressures during CPR, the iNO group had higher CBF than the group treated with HD-CPR alone. Relative to the first minute of CPR, there was more than a two-fold increase in CBF after initiation of iNO. This was likely driven, at least in part, by the higher MAP and thus higher CePP observed with iNO. Local cerebral vasodilation may also have played a role, especially considering the magnitude of the difference in CBF relative to the difference in CePP and the fact that the iNO group continued to have higher CBF in the post-arrest period even in the absence of differential perfusion pressure. Importantly though, previous studies have had varied results regarding the effect of iNO on the cerebral vasculature. For instance, one murine study did not show improvements in post-arrest CBF as measured by continuous arterial spin labeling magnetic resonance imaging.[32] However, another group demonstrated reductions in subarachnoid hemorrhage-associated cerebral vasospasm and dilation of collateral cerebral vessels in a stroke model.[33,34] Regardless of mechanism, the potential for iNO to increase CBF during resuscitation from cardiac arrest, when cardiac output and CBF are typically substantially below normal levels,[35,36] holds great promise as a safe and plausible means of limiting ischemic injury and improving neurologic outcomes.

In previous porcine studies of pediatric IHCA, mitochondrial dysfunction in the brain persisted after successful resuscitation and was associated with neurobehavioral outcomes. As opposed to standard CPR, HD-CPR partly tempered neurologic dysfunction.[17,23] Of particular interest, Complex II-driven respiration was relatively maintained with HD-CPR while Complex I-driven respiration remained depressed.[17] In the present study in which all animals were treated with HD-CPR, oxidative phosphorylation in Complex I

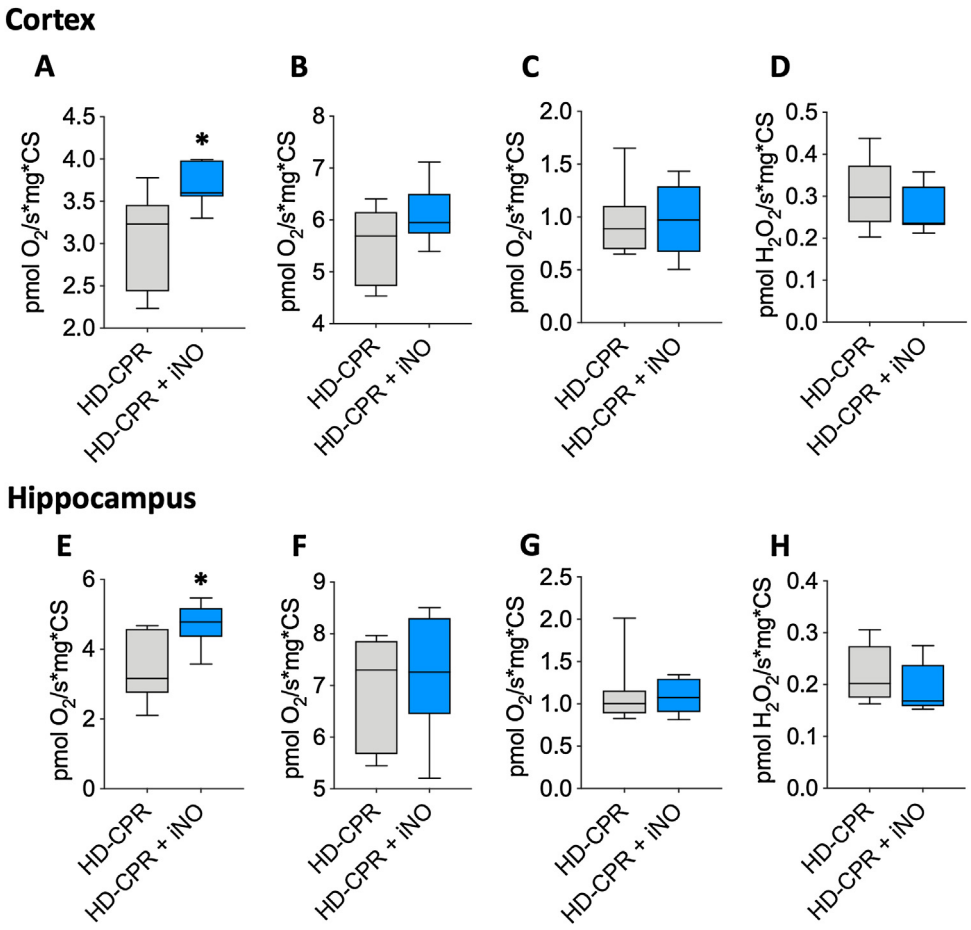

Fig. 2 – Cerebral mitochondrial respiration and reactive oxygen species generation.
Box plots represent measurements from cerebral cortex (top panel) and hippocampus (bottom panel): A **and** E**, Complex I oxidative phosphorylation;** B **and** F**, maximal oxidative phosphorylation (Complex I + Complex II);** C **and** G**, leak respiration; and D and H, mitochondrial reactive oxygen species (Complex I + Complex II). Respiration values are provided in pmol O_2/s mg. Reactive oxygen species are provided in pmol H_2O_2/s mg. Displayed values were normalized to citrate synthase content and were compared between groups using the Wilcoxon rank-sum test. *Indicates statistically significant ($p < 0.05$) difference between groups.**

was higher in animals treated with iNO. This indicates that iNO further enhanced mitochondrial respiratory capacity beyond the effects of the HD-CPR strategy. Complex I (NADH:ubiquinone oxidoreductase) is the largest multimeric enzyme complex of the mitochondrial respiratory chain and its function is particularly susceptible to ischemia-reperfusion injury.[37] The one-hour post-resuscitation assessment of mitochondrial function in this study may have only been equipped to detect differences in Complex I respiration, since Complex I dysfunction is seen early after cardiac arrest in other animal models and other downstream deficiencies in electron transport are not as well characterized at this early timepoint.[38] Our mitochondrial respiration findings build on work by other groups establishing improved mitochondrial function with administration of intravenous nitrites as nitric oxide donors after cardiac arrest.[8,10] While multiple molecular mechanisms related to these effects of nitric oxide have been proposed, it is quite possible that the early differences seen here were a direct result of an attenuated ischemic injury from higher CBF in the iNO group.

Unlike previous investigations by Dezfulian et al. and others,[10,39–41] iNO did not result in decreased mtROS in the hippocampus or cortex in our study, nor did it result in differential cytokine profiles in the systemic circulation or within brain tissue as has been previously observed.[11] These findings could be the effect of differences in the cardiac arrest model, animal species, timing of post-arrest tissue acquisition, brain bioavailability of nitric oxide provided via inhalation as opposed to intravenously administered nitrites, or a host of other factors. Similarly, immunohistochemical evidence of neuronal apoptosis and microglial activation were unchanged with iNO, though this is probably related to the relatively brief duration of post-arrest survival.

This study has limitations. First, these experiments were carried out in previously healthy swine in a controlled environment. Children with IHCA represent a physiologically diverse population whose response to iNO or other resuscitation therapies cannot be predicted from pre-clinical studies alone. Second, intravenous LPS was utilized in this study to simulate pediatric shock-associated IHCA, a relatively common occurrence.[14] Unlike previous work,[12,19] animals did not develop severe shock prior to induction of cardiac arrest, potentially due to age-based differences in LPS response or lower LPS potency.

Table 4 – Histopathology and cytokines.

	HD-CPR with iNO (n = 8)	HD-CPR (n = 8)	*p*
Cerebral histopathology			
TUNEL (n per low-powered field)	2.5 [0.5, 4]	3.5 [0.5, 6.5]	0.68
IBA-1 (semi-quantitative ranking)	1 [1,2]	2.5 [1,3]	0.27
Plasma cytokines[a]			
IL-1 β	6.7 [2.7, 26.9]	16.0 [7.2, 39.5]	0.23
IL-6	213.3 [142.4, 299.6]	229.0 [162.6, 453.5]	0.63
IL-8	796.0 [723.8, 2429.4]	1577.7 [893.9, 2310.8]	0.84
TNF-α	3.53E4 [3.21E4, 4.21E4]	4.08E4 [3.46E4, 4.51E4]	0.23
Cerebral cortex cytokines[a]			
IL-1 β	1.4 [0.6, 1.7]	1.7 [0.8, 2.4]	0.80
IL-6	3.3 [3.1, 3.5]	3.4 [3.2, 3.5]	0.55
IL-8	12.3 [11.6, 17.5]	14.2 [12.2, 27.3]	0.46
TNF-α	9.5 [9.2, 14.7]	13.3 [10.7, 14.7]	0.46
Hippocampus cytokines[a]			
IL-1 β	1.3 [0.7, 1.8]	1.4 [0.9, 2.0]	0.62
IL-6	3.2 [3.1, 3.6]	3.3 [3.3, 3.6]	0.54
IL-8	15.7 [13.8, 17.0]	15.5 [12.3, 17.3]	0.90
TNF-α	12.5 [9.5, 12.9]	13.1 [11.6, 15.0]	0.32

Definition of abbreviations: HD-CPR = hemodynamic-directed cardiopulmonary resuscitation; iNO = inhaled nitric oxide; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling; IBA1 = ionized calcium binding adaptor molecule 1; IL = interleukin; TNF = tumor necrosis factor.
All data displayed as median and interquartile range (in brackets). Histopathology compared using chi-square test. Cytokines compared using Wilcoxon rank-sum test with Bonferroni correction applied within each tissue ($p < 0.0125$ considered statistically significant).
[a] All cytokine concentrations are in pg/mL.

This lessened the overall severity of illness, increased survival rates in the control group, and limited our ability to detect a survival benefit associated with iNO. Third, despite the randomized and blinded nature of the study, measured or unmeasured confounders may have played a role in the physiologic differences observed between groups. For instance, the iNO group received a non-statistically significant higher number of vasopressor doses during CPR. Fourth, our experimental endpoint was 1-h post-arrest survival; future translational studies should examine longer term survival and clinically relevant neurofunctional outcomes. Additional work is also needed to expand our mechanistic understanding of the beneficial effects of iNO, determine its optimal dosing and timing, and better define the characteristics of patients most likely to benefit from it.

Conclusions

In this randomized pre-clinical trial in a large animal model of pediatric IHCA associated with LPS-induced shock, blinded treatment with iNO during and following CPR resulted in superior systemic blood pressures, CePP, rCBF, and cerebral mitochondrial Complex I respiration in the brain. These findings suggest that iNO treatment has beneficial effects during CPR and deserves further investigation as a means of improving neurologic outcomes from pediatric cardiac arrest.

Author contributions

RWM, RMS, ASH, ALR, WPL, VMN, MH, RAB, and TJK contributed to study conception and design. RWM, ASH, ALR, WPL, YL, JS, AR, ND, AMM, MH, and TJK contributed to the acquisition of data. RWM, RMS, ASH, ALR, WPL, JS, AR, ND, CDM, LV, JS, AMM, VMN, MH, RAB, and TJK contributed to the analysis and/or interpretation of data. RWM, RAB, and TJK drafted the manuscript. RWM, RMS, ASH, ALR, WPL, YL, JS, AR, ND, CDM, LV, JS, AMM, VMN, MH, RAB, and TJK contributed to the critical revising of the manuscript. All authors have approved the final version of the manuscript for publication.

Conflicts of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.resuscitation.2021.03.004.

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