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Experimental paper

Effect of vasopressin on brain and cardiac tissue during neonatal cardiopulmonary resuscitation of asphyxiated post-transitional piglets

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ABSTRACT

Background: Epinephrine is currently the only recommended cardio-resuscitative medication for use in neonatal cardiopulmonary resuscitation (CPR), as per consensus of science and treatment recommendations. An alternative medication, vasopressin, may be beneficial, however there is limited data regarding its effect on cardiac and brain tissue following recovery from neonatal CPR.

Aim: To compare the effects of vasopressin and epinephrine during resuscitation of asphyxiated post-transitional piglets on cardiac and brain tissue injury.

Methods: Newborn piglets (n = 10/group) were anesthetized, tracheotomized and intubated, instrumented, and exposed to hypoxia-asphyxia and cardiac arrest. Piglets were randomly allocated to receive intravenous vasopressin (Vaso, 0.4 U/kg) or epinephrine (Epi, 0.02 mg/kg) during CPR until return of spontaneous circulation (ROSC). Left ventricle cardiac tissue, and frontoparietal cerebral cortex and thalamus samples from brain tissue were collected from piglets that survived four hours after ROSC. The concentrations of the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, and tumour necrosis factor (TNF)-α, cardiac troponin-1, lactate, and levels of oxidized and total glutathione were quantified in tissue homogenates.

Main Results: The median time (IQR) to ROSC was 127 (98–162)sec with Vaso and 197 (117–480)sec with Epi (p = 0.07). ROSC rate was 10/10 (100 %) with Vaso and 7/10 (70 %) with Epi (p = 0.21); survival to four hours after ROSC was 10/10 (100 %) with Vaso and 5/7 (71 %) with Epi (p = 0.15). Kaplan-Meier survival curves were significantly different between groups (p = 0.011). Cardiac tissue IL-8 concentration was significantly lower with Vaso than Epi (16.9 (2.94)pg/mg vs. 33.0 (6.75)pg/mg, p = 0.026). All other markers of cardiac and brain tissue injury were similar between Vaso and Epi groups.

Conclusions: Vasopressin is effective in the resuscitation of asphyxiated newborn piglets and is associated with reduced inflammation of the myocardium compared to epinephrine, and there was no evidence of increased tissue injury in the frontoparietal cortex and thalamus regions of the brain. Vasopressin might be a viable alternative to epinephrine during neonatal CPR, but further studies are warranted.

Introduction

The primary goal of cardiopulmonary resuscitation (CPR) following cardiac arrest is to achieve return of spontaneous circulation (ROSC), ensuring sufficient blood flow to organs. CPR is typically achieved through a combination of chest compressions (CC), ventilations, and administration of cardio-resuscitative agents. CPR in the delivery room is rare (~0.06–0.12 % of deliveries), but in the neonatal intensive care unit (NICU) it can occur in 0.25–1 % of patients[1–3]. The infrequent occurrence of advanced neonatal CPR (requiring CC plus epinephrine) combined with the inability to consistently anticipate which newborn infants are at high risk of requiring CPR has contributed to a lack of high-

Abbreviations: CC, Chest compression; CoSTR, Consensus of science and treatment recommendations; CPR, Cardiopulmonary resuscitation; GSSG, Glutathione disulfide; GSH, Glutathione; IQR, Interquartile range; ROSC, Return of spontaneous circulation; SI, Sustained Inflation.

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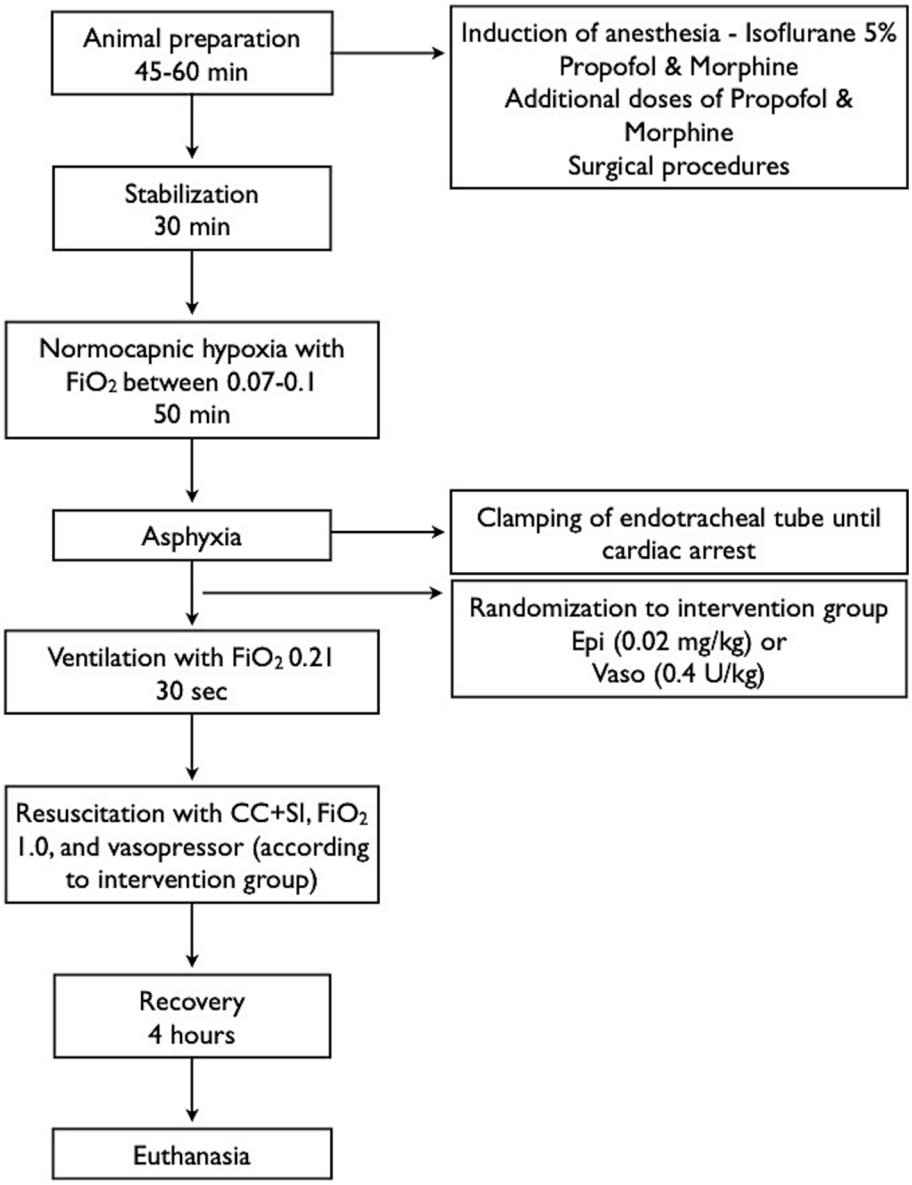

Fig. 1. Study flow diagram.

quality evidence (i.e., large randomized clinical trials) to better guide healthcare providers in resuscitative efforts. Guidelines for neonatal CPR recognize the lack of neonatal data; a recent systematic review from the International Liaison Committee on Resuscitation identified only four cohort studies, including 117 patients, reporting on epinephrine[4]. Consequently, neonatal guidelines extrapolate data from studies with adult patients and animals, which may not apply entirely to neonates[5–6].

Cardiac arrest in newborn infants is mainly a consequence of hypoxia/asphyxia[5], and successful CPR requires delivery of high-quality CC and the most effective cardio-resuscitative agent[7–8]. Current consensus of science and treatment recommendations (CoSTR) state that the cardio-resuscitative agent epinephrine should be given at a dose of 0.01–0.03 mg/kg, preferably intravenously, with repeated doses every 3–5 min during CPR until ROSC[5,9].

Epinephrine causes vasoconstriction in the peripheral vasculature via stimulation of α_1 and α_2 receptors in vascular smooth muscle, and in the heart causes an increase in heart rate, conduction velocity, contractility, and rate of myocardial relaxation (via β_1 receptor stimulations) and leads to smooth muscle relaxation and increased myocardial contractility (via β_2 receptor stimulation)[10–11]. Although the resulting increase in systemic vascular resistance and coronary perfusion pressure aid in organ perfusion during CPR, epinephrine also increases myocardial oxygen demand and the presence of metabolic acidosis can inhibit hemodynamic responses to resuscitation with epinephrine (e.g., aggravated hypertension or tachycardia after ROSC)[12]. Despite epinephrine being used for decades during neonatal CPR, the optimal timing, dose, and route of administration are unknown[10–11,13]. Furthermore, epinephrine administration has been associated with intracranial hemorrhage in premature infants, likely the result of rebound hypertension following ROSC[14].

Vasopressin (also known as anti-diuretic hormone) causes systemic vasoconstriction with pulmonary vasodilation. It functions independently of catecholamine receptor stimulation; thus, its efficacy is not limited by α-receptor downregulation often seen in shock[15–16], does not worsen respiratory and metabolic acidosis and does not increase myocardial oxygen demand[17]. In adults with out-of-hospital cardiac arrest, vasopressin was associated with significantly higher rates of survival as compared with epinephrine until hospital admission (29 % vs. 20 % $p = 0.02$) and hospital discharge (5 % vs. 2 %, $p = 0.04$)[18]. There is limited pediatric and neonatal data regarding vasopressin use

during resuscitation[19]. Given that asphyxia is the primary cause of cardiac arrest in neonates, vasopressin may emerge as a promising alternative to epinephrine[14,20–22].

We aimed to compare the effects of vasopressin and epinephrine during resuscitation of asphyxiated post-transitional piglets on cardiac and brain tissue injury. Our current projects aim to examine if vasopressin is an alternative to epinephrine during neonatal CPR[20–22]; we have published two previous animal studies comparing vasopressin with epinephrine in asphyxiated newborn piglets as well as a trial protocol for an ongoing cluster randomized trial[20–22]. The current study compared vasopressin with epinephrine in asphyxiated newborn piglets with a focus on potential inflammation/injury in the heart and brain. The systemic vasoconstriction and pulmonary vasodilation characteristics of vasopressin may be beneficial in a neonatal resuscitation setting, whereby maintenance of adequate blood flow to the brain and heart may prevent or reduce ischemic injury. Therefore, we hypothesized that vasopressin compared to epinephrine will reduce tissue injury following survival.

Methods

All experiments were conducted after approval from the Animal Care and Use Committee, University of Alberta (AUP00002920), according to the ARRIVE guidelines (refer to appendix for checklist)[23], and registered at preclinicaltrials.eu (PCTE0000528). The study protocol is graphically presented in Fig. 1.

Randomization

Piglets were randomly allocated to either vasopressin (Vaso) or epinephrine (Epi). Allocation was block-randomized 1:1 with variable block sizes using a computer-generated randomization program (http://www.randomizer.org). Sequentially numbered, sealed, brown envelopes containing the group allocation were opened during the experiments (Fig. 1).

Sample size and power estimates

We aimed to compare cardiac and brain tissue injury following the use of epinephrine and vasopressin during neonatal resuscitation. Our previous studies reported a mean (standard deviation (SD)) time to ROSC of 200(20)sec during resuscitation using CC with sustained inflations (CC + SI) and an intravenous epinephrine dose of 0.02 mg/kg[24]. We hypothesized that an intravenous vasopressin dose of 0.4U/kg during resuscitation with CC + SI would reduce time to achieve ROSC and in doing so would reduce tissue injury following survival. A sample size of 20 piglets (10 per group) would be sufficient to detect a clinically important (15 %) reduction in time to achieve ROSC (i.e., 200 vs. 170sec), with 90 % power and a 2-tailed alpha error of 0.05. We used 0.02 mg/kg of epinephrine and 0.4U/kg of vasopressin as these doses were currently or previously recommended.

Blinding

One investigator (TFL) prepared the study drug immediately after opening the randomization envelope. The content of the drug syringe was only known to TFL to conceal group allocation. Another investigator (GMS) assessed and confirmed cardiac arrest and was blinded to group allocation. All other group members were also blinded to group allocation. The statistical analysis was blinded to group allocation, and the investigators were unblinded following completion of the analysis.

Inclusion and exclusion criteria

Newborn mixed breed piglets (0–3 days old) obtained on the day of experimentation from the University Swine Research Technology Centre were included. There was no exclusion criterion.

Animal preparation

Piglets were instrumented as previously described with some modifications[25–26]. Following induction of anaesthesia using isoflurane, piglets were intubated via a tracheostomy, and mechanical ventilation (Sechrist Infant Ventilator Model IV-100; Sechrist Industries, Anaheim, California) was commenced at a respiratory rate of 20 breaths/min with peak inspiratory pressure of 25cmH_2O and positive end expiratory pressure of 5cmH_2O. Oxygen saturation was kept within 90–100 %, and glucose level and hydration was maintained with an intravenous infusion of 5 % dextrose at 10 mL/kg/hr. During the experiment, anaesthesia was maintained with intravenous propofol 5–10 mg/kg/hr and morphine 0.1 mg/kg/hr. Additional doses of propofol (1–2 mg/kg) and morphine (0.05–0.1 mg/kg) were given as needed and body temperature was maintained within normal porcine temperature range of 38.5–39.5 °C using an overhead warmer and a circulating water heat pad[25–26].

Hemodynamic parameters

A 5-French Argyle® (Klein-Baker Medical Inc. San Antonio, Texas) double-lumen catheter was inserted into the right femoral vein for administration of fluids and medications and to measure central venous pressure. A 5-French Argyle® single-lumen catheter was inserted above the right renal artery via the femoral artery for continuous arterial blood pressure monitoring and arterial blood gas measurements. The right common carotid artery was also exposed and encircled with a real-time ultrasonic flow probe (2 mm; Transonic Systems Inc., Ithica, NY) to measure carotid blood flow[25–26]. A 3-lead ECG (Hewlett Packard 78833B monitor, Hewlett Packard Co., Palo Alto, California) using adhesive leads were placed on the skin at the right forelimb, left forelimb, and left hindlimb[25–26].

Piglets were placed in supine position and allowed to recover from surgical instrumentation until baseline hemodynamic measures were stable (minimum of one hour). Ventilator rate was adjusted to keep the partial arterial carbon dioxide (CO_2) between 35 and 45 mmHg, as determined by periodic arterial blood gas analysis. Arterial blood pressure, central venous pressure, heart rate, and percutaneous oxygen saturation were continuously measured and recorded throughout the experiment with a Hewlett Packard 78833B monitor (Hewlett Packard Co., Palo Alto, California)[25–26].

Cerebral oxygenation

Cerebral oxygenation ($crSO_2$) was measured using the Invos™ Cerebral/Somatic Oximeter Monitor (Invos 5100, Somanetics Corp., Troy, MI). The sensor was placed on the right forehead of the piglet and secured with wrap and tape. Light shielding was achieved with a slim cap. The Invos™ Cerebral/Somatic Oximeter Monitor calculates $crSO_2$, which is expressed as the percentage of oxygenated haemoglobin (oxygenated haemoglobin/total haemoglobin). Values of regional oxygen saturation are stored every second with a sample rate of 0.13 Hz[27].

Automated chest compression (CC) machine

The automated CC machine was specifically designed in our laboratory. The CC machine delivers CC rates (50–200/min)[24,28], anterior-posterior chest compression depths (10–70 %)[29–30], acceleration of compressions (100–1000 cm/s^2), speed of recoil (1–100 cm/s), steps per revolution (400–1,200 steps/revolution), and varying duty cycles.

Experimental protocol

Post-transitional piglets were randomized into two groups: epinephrine (Epi, 0.02 mg/kg) and vasopressin (Vaso, 0.4U/kg), which has be used in previous studies[20–21]. Following surgical instrumentation

Table 1
Baseline characteristics.

	Vaso 0.4 U/kg (n = 10)	Epi 0.02 mg/kg (n = 10)	p-value
Age (days)	1.0 (1.0–3.0)	1.5 (0.8–2.0)	0.49
Weight (kg)	2.1 (1.8–2.3)	2.2 (1.9–2.4)	0.45
Heart rate (bpm)	152 (146–198)	161 (139–182)	0.52
Mean arterial blood pressure (mmHg)	65 (60–68)	60 (54–72)	0.59
Carotid blood flow (mL/kg/min)	50 (32–75)	46 (34–86)	0.88
Cerebral oxygenation (%)	41 (40–44)	41 (37–45)	0.71
Arterial pH	7.53 (7.50–7.56)	7.53 (7.44–7.59)	0.84
PaO_2 (torr)	67 (64–73)	72 (68–80)	0.23
$PaCO_2$ (torr)	32 (30–37)	32 (31–35)	0.89
Base excess (mmol/L)	−0.9 (−1.4 ~ -0.3)	−1.1 (−2.0 ~ 0.4)	0.42
Lactate (mmol/L)	3.3 (2.5–4.5)	3.3 (2.6–4.5)	0.88
Hemoglobin (g/dL)	7.7 (6.9–8.9)	7.5 (6.6–8.7)	0.70
Glucose (mmol/L)	8.7 (7.3–10.2)	8.7 (6.5–9.5)	0.82
Body temperature (°C)	39.8 (39.6–40.0)	39.9 (39.6–40.3)	0.68

Data are presented as median (IQR).

Table 2
Characteristics of asphyxia and resuscitation of asphyxiated piglets.

	Vaso 0.4 U/kg (n = 10)	Epi 0.02 mg/kg (n = 10)	p-value
Asphyxia time (sec) †	499 (370–544)	525 (291–600)	0.81
Resuscitation			
Number of drug doses required #	1 (1–1)	1 (1–3)	0.23
Achieving ROSC	10 (100)	7 (70)	0.21
ROSC time (sec) †	127 (98–162)	197 (117–480)	0.07
Survival 4 h after ROSC (% change after ROSC)	10 (100)	5 (71)	0.15

Data are presented as n (%), unless indicated †median (IQR), #median (range); ROSC, return of spontaneous circulation.

and stabilization, piglets were placed onto the automated CC machine, which was placed in the surgical bed. The piglets' anterior-posterior chest diameter was measured from the sternum to the vertebrae touching the bed (anterior to posterior) with a measuring tape and the CC depth of 33 % was calculated[29–30]. Piglets were then exposed to 50 min of normocapnic hypoxia, which was followed by asphyxia. Asphyxia was achieved by disconnecting the ventilator and clamping the endotracheal tube until cardiac arrest. Cardiac arrest was defined as zero carotid blood flow and no audible heartbeat during auscultation. Fifteen seconds after confirmation of cardiac arrest, positive pressure ventilation was provided for 30sec with a Neopuff T-Piece (Fisher & Paykel, Auckland, New Zealand) with 21 % oxygen, peak inspiratory pressure of 30cmH_2O, positive end expiratory pressure of 5cmH_2O, and gas flow of 10 L/min. After 30sec of positive pressure ventilation, mechanical CC were started, with 100 % oxygen and CC during sustained inflation (CC + SI) was delivered with a peak inspiratory pressure of 30cmH_2O for 30sec[26]. The sustained inflation was interrupted for 1sec before a further 30sec of sustained inflation was provided, which was continued until ROSC. The following were the settings of the automated CC machine: CC rate of 90/min, acceleration of compression of 500 cm/s^2, speed of recoil of 50 cm/s, a simulated two-thumb technique, and an anterior-posterior depth of 33 % [24,28–32]. Cardio-resuscitative drug Epi (0.02 mg/kg) or Vaso (0.4U/kg), according to group allocation, was administered intravenously 1 min after the start of CC and thereafter every 3 min until ROSC, with a maximum of three doses and a maximum resuscitation time of 8 min. ROSC was defined as an unassisted heart rate > 100 beats per min for at least 15sec. After ROSC, the piglets recovered for four hours before being euthanized with an intravenous overdose of sodium pentobarbital (120 mg/kg). During the 4-hour recovery period

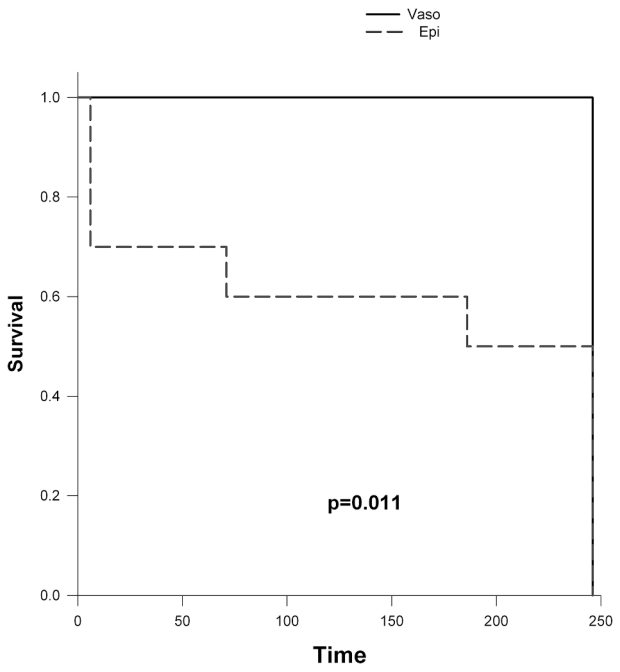

Fig. 2. Kaplan-Meier survival curve showing the survival of piglets treated with Vaso or Epi during resuscitation. Time (min) is post-return of spontaneous circulation (ROSC). In the Epi group, 3/10 piglets did not achieve ROSC; two of the remaining piglets that achieved ROSC did not survive the 4-hour recovery period. In the Vaso group, 10/10 piglets achieved ROSC and survived the 4-hour recovery period.

anaesthesia was maintained with intravenous propofol 5–10 mg/kg/hr and morphine 0.1 mg/kg/hr, oxygen saturation was kept within 90–100 %, and glucose level and hydration was maintained with an intravenous infusion of 5 % dextrose at 10 mL/kg/hr. If there was no ROSC at the end of the resuscitation time, piglets were euthanized immediately with an intravenous overdose of sodium pentobarbital (120 mg/kg). Autopsies were performed in all piglets to assess for injuries to the sternum, ribs, heart, or lungs (e.g., bruising, abrasions, contusions, fractures).

Tissue preparation and analysis

Tissue samples were only collected from piglets that survived four hours after ROSC. Following euthanasia, left ventricular cardiac tissue and brain tissue was collected. Cardiac tissue samples were snap frozen in liquid nitrogen and stored at −80 °C. The whole brain was removed from the skull and placed in ice-cold 2-methylbutane for 10 min before being stored at −80 °C. Areas of the frontoparietal cerebral cortex and thalamus were isolated from the whole brain and used for tissue analysis. Tissue samples were homogenized in lysis buffer (0.5 % Tween-20/PBS containing protease inhibitor cocktail), centrifuged (3,000xg for 10 min at 4 °C), the supernatants were collected, and protein concentration was quantified using the Bradford method. Evidence of cardiac and/or brain injury was determined by quantification of the concentrations of the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, and tumour necrosis factor (TNF)-α in tissue homogenates by using commercially available ELISA kits as per manufacturer's instructions (PLB00B, P6000B, P8000, PTA00; R&D Systems, Minneapolis, MN). The concentration of cardiac troponin-1 was determined in cardiac tissue homogenates using a commercially available ELISA kit (EKX-FB6XG6-96, Nordic BioSite, Sweden). Cytokine and troponin-1 concentrations were expressed relative to protein concentration. Lactate and levels of oxidized and total glutathione (GSSG and GSH, respectively) in the tissue homogenates were used as surrogate markers for hypoxic damage and oxidative stress. Tissue lactate levels in cardiac and brain tissue samples were determined by a nicotinamide adenine nucleotide enzyme coupled colorimetric assay, as previously described[33]. Tissue response to

Table 3
Blood gas changes and carotid blood flow before and after resuscitation.

	Vaso 0.4 U/kg	Epi 0.02 mg/kg	p-value
Commencement of resuscitation			
Heart rate (bpm)	0	0	1.00
Mean arterial blood pressure (mmHg)	8 (6–10)	6 (5–10)	0.37
Carotid blood flow (mL/kg/min)	0	0	1.00
Arterial pH	6.70 (6.60–6.81)	6.71 (6.59–6.78)	0.82
PaO_2 (torr)	14 (11–17)	11 (6–18)	0.44
$PaCO_2$ (torr)	84 (76–97)	88 (68–113)	0.67
Base excess (mmol/L)	−25 (−27 ~ −23)	−26 (−29 ~ -21)	0.96
Lactate (mmol/L)	19 (17–20)	18 (16–20)	0.49
Hemoglobin (g/dL)	9.1 (7.7–10.4)	8.7 (7.3–9.4)	0.70
Glucose (mmol/L)	15.9 (13.7–17.6)	15.7 (11.7–19.1)	0.82
Body temperature (°C)	39.6 (39.5–39.8)	39.6 (39.5–39.7)	0.79
4-hours after return of spontaneous circulation			
Heart rate (bpm)	249 (187–256)	178 (166–194)	0.02
Mean arterial blood pressure (mmHg)	39 (29–53)	35 (29–42)	0.40
Carotid blood flow (mL/kg/min)	23 (18–49)	19 (8–42)	0.46
Arterial pH	7.39 (7.32–7.47)	7.06 (7.01–7.31)	0.004
PaO_2 (torr)	64 (48–71)	72 (45–87)	0.93
$PaCO_2$ (torr)	33 (30–37)	35 (30–49)	0.32
Base excess (mmol/L)	−3.9 (−9.1 ~ -1)	−18 (–23 ~ -9)	0.005
Lactate (mmol/L)	5.8 (4.6–7.9)	15.4 (7.7–17.6)	0.005
Hemoglobin (g/dL)	9.0 (7.0–10.2)	8.5 (6.9–8.8)	0.35
Glucose (mmol/L)	10.9 (8.3–14.4)	10.5 (10.3–11.5)	0.57
Body temperature (°C)	39.7 (39.5–39.9)	39.4 (39.3–39.9)	0.59

Data are presented as median (IQR).

oxidative stress was measured by determining GSSG and GSH using a commercially available assay kit as per manufacturer's instructions (703002, Cayman Chemical, Ann Arbor, MI) and the ratio of GSSG/GSH was calculated.

Data collection and statistical analysis

The demographics of the study piglets were recorded. Transonic flow probe, heart rate and pressure transducer outputs were digitized and recorded with the LabChart® programming software (ADInstruments, Houston, Texas). Hemodynamic data until time to ROSC and post-resuscitation was analyzed (i.e., arterial blood pressure, central venous pressure, carotid blood flow). Data are presented as mean (standard deviation – SD) for normally distributed continuous variables and median (interquartile range – IQR) when the distribution was skewed. Data were tested for normality (Shapiro-Wilk and Kolmogorov-Smirnov test) and compared using either Student-T-Test (data normally distributed) or Rank Sum if data were skewed. *P*-values are 2-sided and $p < 0.05$ was considered statistically significant. Statistical analyses were performed with SigmaPlot (Systat Software Inc, San Jose, California).

Results

Twenty mixed breed newborn post-transitional piglets 0–3 days old, ranging in weight from 1.8-2.4 kg, were obtained on the day of the experiment and were randomly assigned to Vaso (0.4 U/kg; n = 10) or Epi (0.02 mg/kg; n = 10). There were no differences in baseline parameters between groups (Table 1).

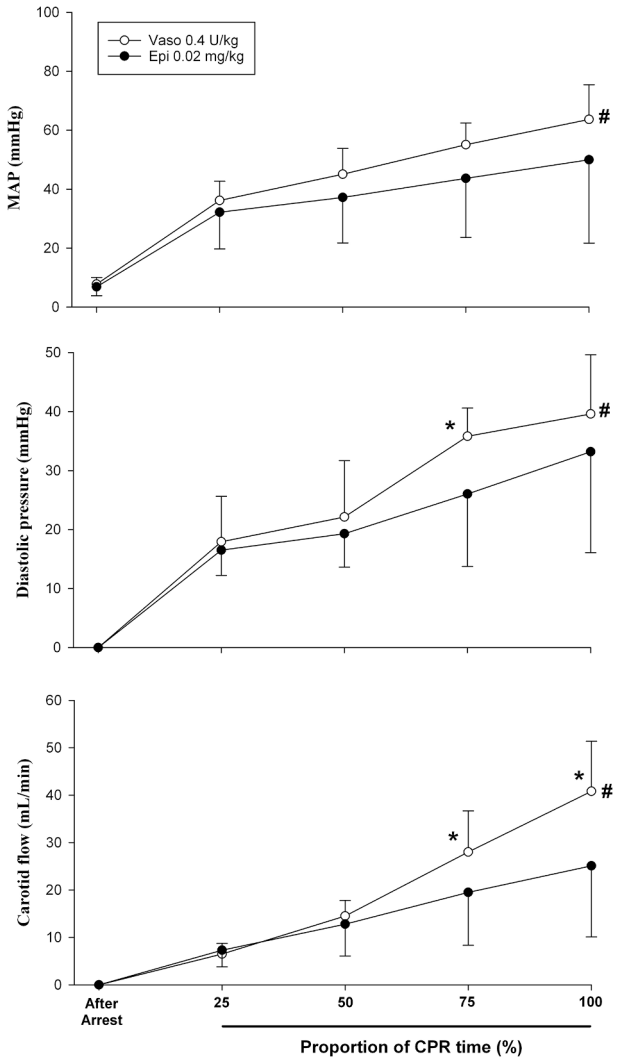

Fig. 3. Hemodynamic changes during resuscitation; mean (SD) data for mean arterial pressure (MAP), diastolic blood pressure, and carotid blood flow are presented as mean (SD) and parameters are plotted against the proportion of the cardiopulmonary resuscitation (CPR) time, expressed as a percentage of the total CPR time. #$p < 0.05$ vs. Epi group over time; *$p < 0.05$ vs. Epi group at respective time point.

Resuscitation outcomes

Table 2 presents a summary of asphyxia and resuscitation outcome measures. The median (IQR) duration of asphyxia before commencement of chest compressions was not different between groups: 499 (370–544)sec with Vaso and 525 (291–600)sec with Epi ($p = 0.81$). The median (IQR) time to achieve ROSC was 127 (98–162)sec with Vaso and 197 (117–480)sec with Epi ($p = 0.07$). The number of piglets that achieved ROSC was 10/10 (100 %) with Vaso and 7/10 (70 %) with Epi ($p = 0.21$). The proportion of piglets that survived 4 h after ROSC was 10/10 (100 %) with Vaso and 5/7 (71 %) with Epi ($p = 0.15$). Fig. 2 presents the Kaplan-Meier survival curves from Vaso and Epi groups; there was a significant difference in survival between piglets treated with Vaso during resuscitation compared to Epi ($p = 0.011$).

Hemodynamic parameters

Hemodynamic parameters at baseline (Table 1) and at commencement of resuscitation (Table 3) were not different between groups. Hemodynamic changes during resuscitation are presented in Fig. 3.

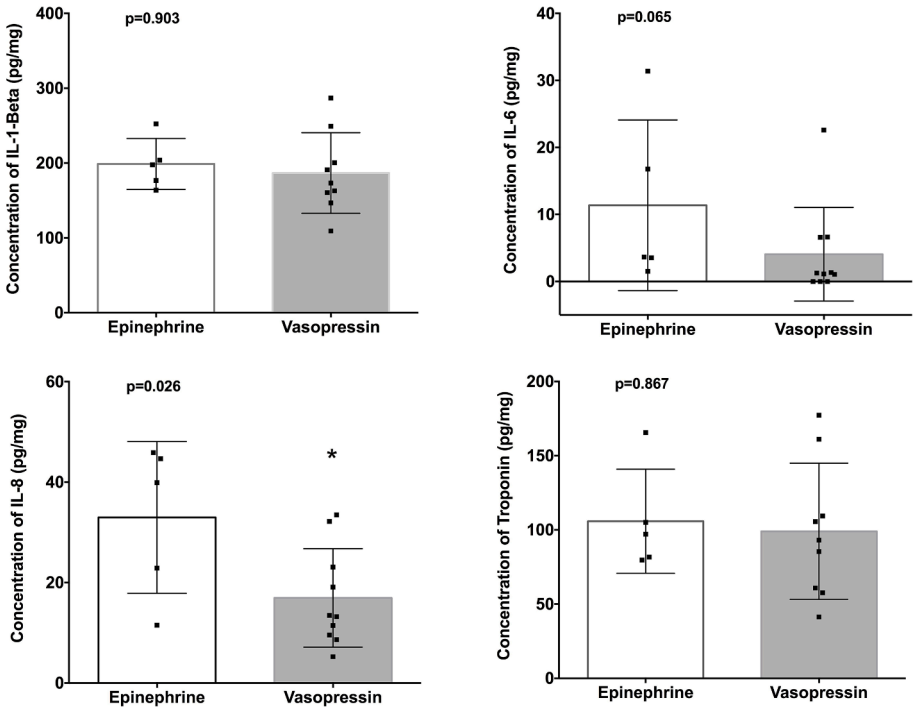

Fig. 4. Left ventricle cardiac tissue injury markers presented as mean (SD), the squares represent the concentration of each individual piglet. *p < 0.05 vs. Epi group.

Throughout the resuscitation, piglets treated with Epi demonstrated significantly lower mean arterial pressure (MAP), diastolic blood pressure, and carotid blood flow ($p < 0.05$) compared to the Vaso group (Fig. 3). At the end of the 4-hour recovery period, piglets treated with Vaso during resuscitation had significantly higher heart rate, arterial pH, base excess, and lower plasma lactate levels compared to piglets treated with Epi (Table 3), indicating less metabolic acidosis and less severe hypoxemia in the Vaso group compared to Epi group.

Cardiac tissue injury markers

The concentrations of cardiac tissue injury and pro-inflammatory markers concentrations are presented in Fig. 4 and are expressed as mean (SD). There was no significant difference between Vaso and Epi groups in the concentration of the pro-inflammatory cytokines IL-1β (186.7 (21.5)pg/mg vs. 198.8 (10.7)pg/mg, $p = 0.913$) and IL-6 (4.06 (2.21)pg/mg vs. 11.36 (5.59)pg/mg, $p = 0.065$). The concentration of IL-8 in cardiac tissue was significantly lower in the Vaso group compared to Epi (16.9 (2.94)pg/mg vs. 33.0 (6.75)pg/mg, $p = 0.026$). TNF-α concentrations were too low to be detected in cardiac tissue samples from both Vaso and Epi groups. Cardiac troponin I (an indicator of myocardial damage), cardiac tissue lactate (a marker for tissue perfusion and anaerobic metabolism), and GSSG/GSH ratio (an indicator of tissue response to oxidative stress) were not significantly different between Vaso and Epi groups.

Brain tissue injury markers

Brain tissue injury marker concentrations are presented in Figs. 5a and 5b and are expressed as mean (SD). There was no significant difference between Vaso and Epi groups in the concentration of the pro-inflammatory cytokines IL-1β, IL-6, and IL-8 in frontoparietal cortex tissue samples (Fig. 5a). Similarly, there were no significant differences in pro-inflammatory cytokine concentration in thalamus tissue samples (Fig. 5b) between Vaso and Epi groups. TNF-α concentrations were too low to be detected in frontoparietal cortex and thalamus tissue samples from both Vaso and Epi groups. Tissue lactate and GSSG/GSH ratio in both the frontoparietal cortex and the thalamus were not significantly different between Vaso and Epi groups.

Discussion

In the current study, we investigated markers of cardiac and brain tissue injury following neonatal resuscitation with epinephrine compared to vasopressin in a post-transitional asphyxiated piglet model. The results of our study can be summarized as follows: 1) time to achieve ROSC and number of piglets achieving ROSC was not different (Table 2), 2) vasopressin significantly improved hemodynamic responses during resuscitation (Fig. 3) and post-resuscitation survival (Fig. 2, Kaplan-Meier survival curves), 3) vasopressin significantly improved blood gas parameters at 4 h after ROSC (Table 3) indicative of less severe metabolic acidosis, 4) vasopressin-treated piglets had significantly lower levels of the pro-inflammatory marker IL-8 in left ventricular cardiac tissue (Fig. 4), and 5) vasopressin did not lead to significantly increased markers of brain injury compared to epinephrine.

The goal of resuscitation is to prevent death and minimize long-term neurodevelopmental impairments. Both the hypoxic/ischemic event and the reoxygenation-reperfusion following resuscitation can trigger a cascade of inflammatory and oxidative stress pathways that may result in injury to the brain. Time to ROSC is a key factor that can affect outcomes in asphyxiated infants, therefore optimizing ventilation and hemodynamics during CPR to achieve a faster recovery can reduce inflammation and oxidative stress and improve long-term outcome[34–36]. In the current study we observed no significant difference in time to ROSC, however we did observe significantly improved hemodynamic responses during resuscitation and improved survival (4-hours post-ROSC) after resuscitation with vasopressin-treated compared to epinephrine-treated piglets, as well as less severe metabolic acidosis in recovery.

To our knowledge, this is the first animal study that has investigated cardiac and brain tissue injury markers following resuscitation with cardio-resuscitative agents vasopressin and epinephrine. Through it's β-adrenergic receptor stimulation, epinephrine increases myocardial oxygen demands, respiratory and metabolic acidosis, and inhibits

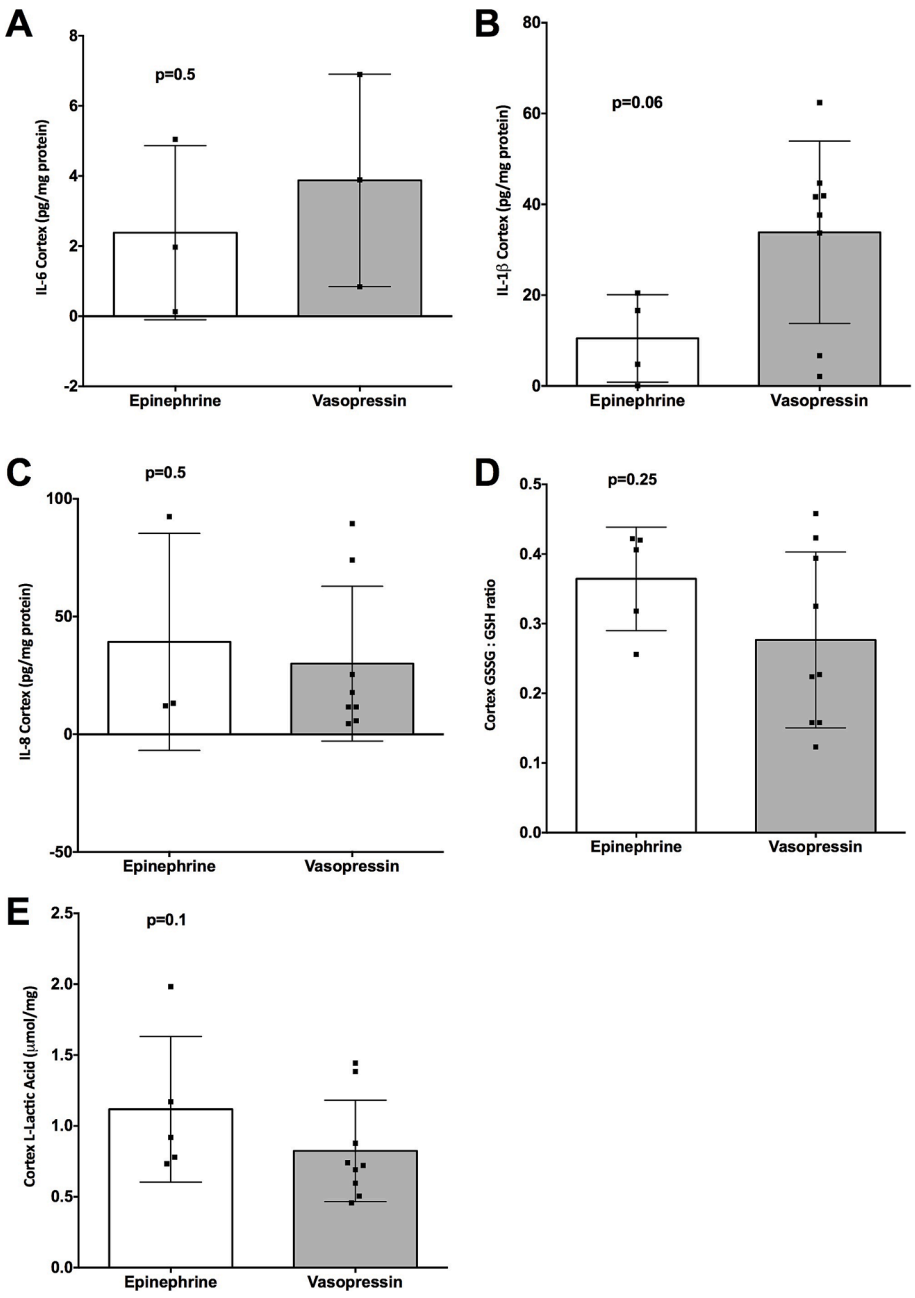

Fig. 5a. Frontoparietal cortex tissue injury markers presented as mean (SD), the squares represent the concentration of each individual piglet.

hemodynamic responses, which can lead to aggravated hypertension or tachycardia after ROSC[10–11]. Indeed, epinephrine administration has been associated with intracranial hemorrhage in premature infants, likely the result of rebound hypertension following ROSC[14]. Owing to these adverse effects of epinephrine administration it was imperative to examine cardiac and brain injury following vasopressin and epinephrine use in neonatal resuscitation. Oxidative stress related injury has also been reported with high-dose of epinephrine and in our previous experiments of prolonged epinephrine infusion in piglets with hypoxia and reoxygenation[37]. In hypoxic piglets with shock and hypotension during reoxygenation, low-dose vasopressin was found to have less oxidative stress-related injury when compared to placebo-controls[38]. Asphyxia is an inflammatory event with activated Src homology 2 domain–containing protein tyrosine phosphatase (SHP) pathways, in addition to the tissue injury due to hypoxia–ischemia and oxidative stress following reoxygenation and reperfusion. While epinephrine at high doses may induce oxidative stress[39], vasopressin has been reported to have anti-inflammatory actions[40–41].

Previously we have reported a significant increase in post-resuscitation carotid artery blood flow in piglets treated with vasopressin during neonatal resuscitation[21]. Post-resuscitation hypertension, although present in both vasopressin and epinephrine-treated piglets, was observed in vasopressin-treated piglets for two-times longer than epinephrine-treated piglets. Although there is concern for the effects post-resuscitation hypertension could have on cerebral tissue injury, with the exemption of IL-1β which was substantially higher in the cortex region with vasopressin, no increased injury in the frontoparietal cortex and thalamus regions of the brain were observed compared to epinephrine. The similarity between vasopressin and epinephrine in terms of pro-inflammatory cytokine expression, lactate tissue levels, and GSSG/GSH ratio in brain tissues four hours after achieving ROSC indicates an aspect of safety for vasopressin as a cardio-resuscitative agent.

Left ventricular cardiac tissue expression of the pro-inflammatory cytokine IL-8 was significantly higher in the epinephrine-treated

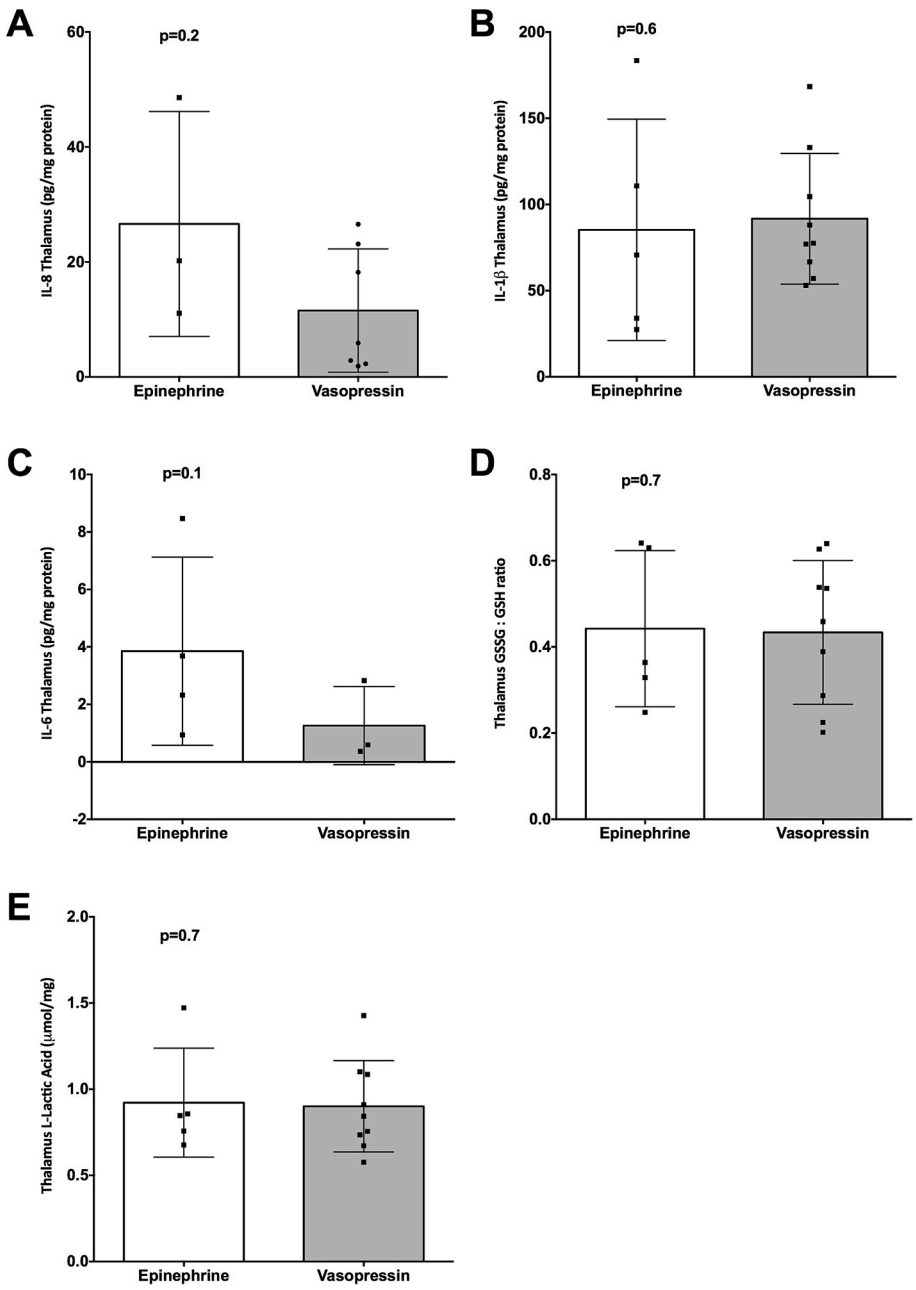

Fig. 5b. Thalamus tissue injury markers presented as mean (SD), the squares represent the concentration of each individual piglet.

piglets compared to vasopressin-treated. IL-8 is involved in inflammation through its ability to attract and activate neutrophils to sites of inflammation. Ischemia reperfusion injury following resuscitation has been shown to be closely related to neutrophils activated by pro-inflammatory cytokines, including IL-8[42]. Not only are neutrophils essential for initiating inflammation, but their prolonged presence can lead to ongoing cardiac inflammation and increased reactive oxygen species. Ito *et al* showed that serum IL-8 levels increased after ROSC in resuscitated cardiac arrest patients; patients who died or became brain dead had significantly higher IL-8 levels than other patients[43]. Elevation of the serum IL-8 level was related to the total dose of epinephrine administered during resuscitation, as well as to peripheral neutrophil counts. The increase in tissue IL-8 concentrations in the epinephrine group in the current study may be indicative of myocardial inflammation, and vasopressin use during resuscitation may reduce the risk of developing post-ROSC inflammation.

Limitations

In the current study, we administered cardiopulmonary resuscitation using continuous CC during sustained inflation (CC + SI), which although is mentioned in the "knowledge gap" section of the neonatal resuscitation guidelines[5], is not the current recommended clinical practice[6]. Our use of a piglet asphyxia model is a strength of this translational study, as this model closely simulates delivery room events, with the gradual onset of severe asphyxia leading to bradycardia and eventual cardiac arrest. A further strength of this study is the use of our custom-designed automated CC machine, which allowed consistent delivery of CC rates and reduced potential bias (e.g., fatigue during CC, or inability to constantly achieve rate and/or depth of CC)[24,28–30]. Our asphyxia model uses piglets that have already undergone the fetal-to-neonatal transition, were sedated/anesthetized, and uses tracheostomy with a tightly sealed endotracheal tube to prevent leak, which does not occur in the delivery room, and are limitations of our model[44–45]. We

only observed the piglets for 4 h post ROSC, which might not be enough to assess the full development of inflammation and effects of inflammation. Moreover, as piglets were euthanized after a 4-hour recovery period, we were unable to analyze long-term survival or neurological function outcomes.

Conclusions

Vasopressin improves post-resuscitation survival and is associated with reduced inflammation of the myocardium compared to epinephrine. There was no evidence of increased tissue injury in the frontoparietal cortex and thalamus regions of the brain following the use of vasopressin versus epinephrine in neonatal resuscitation. Vasopressin might be a viable alternative to epinephrine during neonatal resuscitation and further studies are warranted.

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CRediT authorship contribution statement

Ali Chaudhry: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Megan O'Reilly:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marwa Ramsie:** Writing – review & editing, Methodology, Investigation, Data curation. **Tze-Fun Lee:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Po-Yin Cheung:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Georg M. Schmölzer:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

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