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Neurologic Outcome after Asphyxial Cardiac Arrest in a Juvenile Porcine Model: Comparison of Epinephrine and Vasopressin, Alone or Combined with Nitroglycerin

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NL, TX, LP, ID and EK designed the research. Authors NL, GV, TL, SG, GA, TX, LP, ID and DP performed the research. Authors NL, TX, TMK and GV contributed new reagents/analytic tools. Authors NL, NK, GA and TMK analysed the data. Authors NL, TMK, NK and ID wrote the paper. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Hypoxemic encephalopathy is a devastating complication of asphyxial cardiac arrest in children, commonly occurring despite prompt resuscitation. Epinephrine, incorporated in present algorithms, may contribute to unfavorable outcome by causing excessive vasoconstriction, but the effects of alternative agents are unclear. Here, we compared the neurologic outcome after epinephrine with that after vasopressin (alone or combined with nitroglycerin) in a juvenile porcine model of asphyxia.

Study Design: Randomized experimental animal study.

Place and Duration of Study: Experimental surgery and surgical research department, of the Medical School, Athens University, from January 2013 to February 2016.

Methodology: Asphyxia was induced in 30 Landrace piglets (12-15 weeks of age) by occlusion of the endotracheal tube, leading to cardiac arrest. Four minutes thereafter, resuscitation was commenced with mechanical ventilation and chest compressions. The animals were randomized into three treatment groups, namely into epinephrine (E, n=10) vasopressin (VP, n=10) or vasopressin plus nitroglycerin (VP+NTG, n=10). Hemodynamic variables were measured at baseline and for 30 minutes after the onset of resuscitation. Neurological deficit and brain histological damage scores were assessed in survivors at 24 hours.

Results: At baseline, hemodynamic variables did not differ between groups. The rates of restoration of spontaneous circulation (ROSC), followed by successful extubation, were comparable in the three groups, as were 24-hour survival rates. Mean aortic pressure and coronary perfusion pressure were higher in the VP and VP+NTG groups at the 5th minute of resuscitation, but lower than in the E group at the 30th minute. Neurological deficit and brain histological damage were improved after VP or VP+NTG, compared to that after E.

Conclusion: In this juvenile porcine model of asphyxial cardiac arrest, vasopressin (with or without nitroglycerin) yielded improved neurologic outcome, when compared to epinephrine, albeit similar ROSC and survival rates.

Keywords: Asphyxia; cardiac arrest; resuscitation; outcome; vasopressin; nitroglycerin.

1. INTRODUCTION

Asphyxia constitutes the most common cause of cardiopulmonary arrest (CA) in pediatric populations, invariably caused by foreign body aspiration [1,2,3]. Asphyxial CA is characterized by progressive hypoxemia and hypercapnia, leading to rhythm disturbances and circulatory failure [4]. As the brain is the most vulnerable organ regarding oxygen demand [4,5], resuscitation is complicated by hypoxic encephalopathy in as high as 50% of survivors [3]. Due to this ominous outcome, refinement of resuscitation algorithms toward efficient maintenance of brain perfusion has been at the center of numerous research efforts [6].

Epinephrine, an endogenous catecholamine with potent alpha- and beta-adrenergic actions, remains the agent of choice in advanced life support algorithms, incorporated in international guides [5]. However, its use has been challenged by animal data, showing microcirculatory impairment by epinephrine [7], which results in post-resuscitation myocardial dysfunction [8] and poor neurologic outcome [9].

Vasopressin, an endogenous hormone inducing systemic vasoconstriction, can improve the return of spontaneous circulation (ROSC) after prolonged advanced life support [10,11,12]. However, despite the accumulated knowledge from experimental [13,14] and clinical [15] data, there is insufficient evidence to support its use as an alternative to epinephrine in asphyxial CA in pediatric populations [10].

In an effort to reduce the adverse effects of vasoconstictive agents, previous reports indicated that the addition of nitroglycerin may increase survival rates in comparison with vasopressin alone [16,17]. Nitroglycerin induces vasodilatation in the systemic, pulmonary and coronary circulation, and can increase cardiac output [17]. Despite these results, favoring the use of nitroglycerin in resuscitation, data on neurologic outcomes are scarce.

In the present study, we compared hemodynamic responses, neurological outcome and survival after three randomly assigned regimens, namely epinephrine versus vasopressin versus vasopressin combined with nitroglycerin, in a juvenile asphyxia porcine model.

2. MATERIALS AND METHODS

2.1 Animal Study Population and Ethics

The study was conducted on 30 domesticated landrace/large white piglets (all male, 12-15 weeks of age, weighing 20±2 kg). The experimental protocol was approved by the General Directorate of Veterinary Services (permit no. K/3038), and all experimental procedures conformed to European legislation (European Union directive for the protection of animals used for scientific purposes, as revised in 2010/63/EU). All animals were supplied by the same breeder and were of conventional microbiological status. No signs of disease were present after veterinarian clinical examination. They were housed in singles, in cages with an area of 1 m^2. A 12 h/12 h light/dark cycle was provided in climate-controlled conditions, at a temperature of 22±2°C and relative humidity of ~55%. The animals were given free access to water and standard, commercially available food; they were acclimatized to the laboratory conditions for a one week prior to the experiments.

Prior to the experimental procedure, the piglets were randomized (with the use of a sealed envelope) in three groups, each of n=10 animals, namely into epinephrine (E), vasopressin (VP), and vasopressin plus nitroglycerin (VP-NTG). Treatment was administered in the three groups as follows: epinephrine (0.02 mg/kg, diluted in 10 ml saline, as bolus injection), vasopressin (0.4 IU/kg, diluted in 10 ml saline, as bolus injection), or vasopressin (0.4 IU/kg /10 ml dilution, as bolus injection) plus nitroglycerin (7.5 μg/kg). To ensure the blinded conduct of the study, treatment was administered by an investigator, who did not participate further in the specific experiment.

2.2 Experimental Protocol

The animals were pre-medicated with an intramuscular injection of ketamine hydrochloride (10 mg/kg), midazolam (0.5 mg/kg), and atropine sulfate (0.05 mg/kg). After a period of 15 min, the pigs were transported to the operating room. All procedures, described below, were conducted under aseptic conditions. Intravenous access was attained via cannulation of the lateral auricular veins bilaterally (BD Venflon 20 GA 1.26IN 67 ml/min). Anesthesia was induced with an intravenous bolus dose of propofol 1% (2 mg/kg) and fentanyl (2 μg/kg). Intubation was performed with an endotracheal cuffed-tube (MLT 4.5 or 5.0 Oral 27 mm Mallinckrodt Medical). Additional propofol 1 mg/kg, rocuronium 1 mg/kg were administered before connecting the animals to the automatic volume-controlled ventilator (ventiPac Sims PneuPac) with oxygen (FiO_2 21%) and total tidal volume of 15 ml/kg to maintain normocapnia. End-tidal CO_2 (Nihon Kohden Corp.) and pulse oximetry (SpO_2) (Vet/Ox Plus 4700) were continuously monitored, with the sensor placed on the tongue of the intubated animal. Anesthesia was maintained by infusion of propofol 5 mg/kg/h, remifentanyl (20 μg/kg/h) and rocuronium (0.3 mg/kg/h.). Intravenous chemoprophylaxis with kefuroxime 750 mg was administered to prevent infection.

A six-limb electrocardiogram (ECG) was continuously monitored (Mennen Medical Model 6523), and heart rate was determined from the ECG signal. The right internal jugular vein was surgically exposed and a 5.5 F catheter (Opticath, Abbott) was advanced into the right atrium. For monitoring of the aortic pressure, a 5F catheter was placed in the ascending aorta via the internal carotid artery, permitting the recording of systolic and diastolic aortic pressure (Model 6523, USCI CR, Bart, Papapostolou, Greece); mean aortic pressure was determined by the electronic integration of the aortic blood pressure waveform. Coronary perfusion pressure (CPP) was calculated as the difference between aortic and the simultaneously measured right atrial pressures.

2.3 Asphyxia Protocol

The experimental asphyxia protocol, followed here, has been described previously [18]. In brief, after collection of baseline data, the endotracheal tube was clamped at the end of a normal exhalation and the piglet was asphyxiated until cardiac arrest. Any form of gasping was prevented by full muscle paralysis, and all infusions were stopped. Asphyxial cardiac arrest was defined as a mean aortic pressure (MAP) below 10 mmHg and by the absence of aortic pulsation; at this time-point, the endotracheal tube was unclamped. CA-induction time was defined as the time from clamping until CA, as defined above, whereas untreated CA-time, defined as the time-interval between onset of CA and the start of CPR, was set at 4 min. The total asphyxia time-interval was defined as the period between clamping and the onset of resuscitation. The resuscitation efforts included mechanical

ventilation with inspired oxygen (at a concentration of 100%), adjusted to obtain partial pressure of end-tidal CO2 ($PETCO_2$) of 35-40 mm Hg. Mechanical chest compressions were commenced, at a rate of 100/min (LUCAS Chest Compression System), followed by the intravenous administration of drugs to the auricular vein, and, finally, by a 10 ml saline flush.

Two minutes after the onset of chest compressions, advanced life support (ALS) was commenced guided by the underlying cardiac rhythm, according to current consensus [12]. Specifically, when ventricular fibrillation (VF) was present, defibrillation was attempted with 4J/Kg monophasic waveform shock (Medical Research Laboratories, Inc, Porta Pac 190), via 12 cm adhesive electrodes. In case of defibrillation-failure, chest compressions were continued for 2 min, and defibrillation was repeated. Successful ROSC was defined as MAP of 50 mmHg or above, for a minimum period of 10 min, as previously [19]. The endpoints of the experiment were defined as (a) return of ROSC or (b) asystole or pulseless electrical activity (PEA) after three cycles of CPR or (c) persisting VF, after the third defibrillation. All piglets received normal saline intravenous infusion for post-resuscitation circulatory support. Arterial blood samples were taken before the induction of cardiac arrest, 1 min before CPR and 30 min after ROSC.

As in previous animal studies [18], all successfully resuscitated animals were monitored for 30 min, while light anesthesia was maintained. No antiarrythmic or additional vasoconstrictive agents were administered after ROSC. In the surviving animals, all catheters were removed and the blood vessels were ligated. The ventilator circuit was disconnected from the endotracheal tube, while manual ventilation with 100% oxygen was continued by squeezing a reservoir bag. In animals displaying spontaneous respiration, the tracheal tube was connected to a T-tube for oxygen administration. After administration of atropine (0.2 mg/kg) and neostigmine (0.05 mg/kg) the animals were extubated, as soon as spontaneous respiration was deemed adequate. Oxygen was given by face mask and the animals were further observed for 15 min. Successfully resuscitated animals were returned to their cage and received paracetamol every 12 hours, with free access to food and water. If weaning of mechanical ventilation failed, the animals were euthanized and autopsied.

The animals were observed for 24 hours after the onset of CA. At the end of this period, the neurologic status was evaluated using the neurologic alertness scores [20], by an investigator blinded to treatment allocation. The total score consisted of 5 components: *posture* (if the animal can stand, attempt to stand, or if it is lying on the side), *gait* (normal, ataxic, or absent), *response to stimuli* (response to all stimuli, only to painful stimuli, or no response), *pupils* (normal, anisocoria, or mydriasis) and *convulsion* (absent, tonic-clonic, or generalized). The NDS scoring-system adds to a score of 100, assigned for complete recovery, whereas a score of 0 is assigned for brain-dead status; thus, higher NDS-values indicate better outcome.

At the end of the experiment, the animals were euthanized with an overdose of sodium thiopental, after sedation with ketamine 10 mg/Kg and midazolam 0.5 mg/Kg. Subsequently, the pigs were necropsied, with special attention given to the presence of rib-cage injury or internal organ damage. The brain was removed from the skull and immersed in 10% paraformaldehyde for 72 hours; it was then cut in a series of 0.4 μm-thick slices, each stained with hematoxylin–eosin. Sections of four brain regions (frontal and temporal cortex, hippocampus and cerebellum) were evaluated by light microscopy for ischemic neuronal changes, capillary congestion and edema. The score was assessed on a four-point scale, as follows: minimal = 1; moderate = 2; severe = 3; and maximal = 4. The severity score was then multiplied by a weighing factor, depending on the type of lesion (i.e., edema×1, ischemic neuronal change×4 and capillary congestion×1). The total brain histological damage score (HDS) was the sum of all 4 area scores, as previously suggested [21,22]. The flow chart of the experimental protocol is graphically depicted in Fig. 1.

2.4 Statistical Analysis

All analyses were carried out using the statistical package SPSS vr 17.00 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, Ill., USA). The Kolmogorov-Smirnov test was utilized for normality analysis. Data are expressed as mean±standard deviation (S.D.) or median (in case of violation of normality) for continuous variables and as percentages for categorical variables.

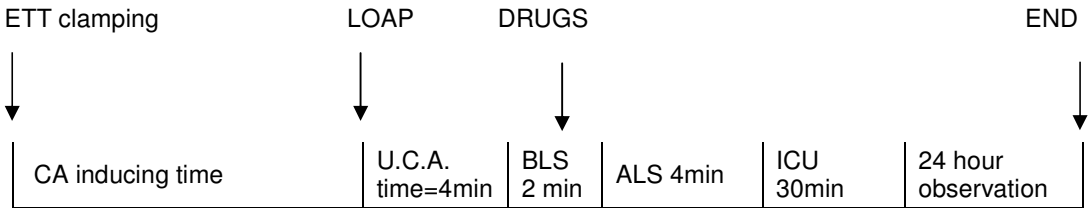

Fig. 1. Experimental protocol timetable

ETT: Endotracheal tube clamping, LOAP: Loss of aortic pulsations, UCA: Untreated cardiac arrest time, BLS: Basic life support, ALS: Advanced life support algorithms, ICU: Intensive care unit, Total asphyxial time interval: CA inducing time+untreated cardiac arrest time

Continuous variables were examined using the one way analysis of variance, followed by pair-wise, between-groups comparisons with the post-hoc Bonferroni test. Kruskal-Wallis test and Mann-Whitney U-test were used in case of violation of normality. Statistical significance was set at an alpha value of .05.

3. RESULTS

3.1 Pre-arrest Period

Baseline hemodynamic measurements did not differ between-groups, as shown in Table 1.

3.2 Asphyxial Period

At baseline, all animals were in normal sinus rhythm, whereas sinus tachycardia was noted during the initial four minutes after clamping. The total asphyxia time interval was comparable between groups, i.e., 13.18 ±1.68 min for the E group, 12.97±1.42 min for the VP group and 12.80±1.88 min for the VP+NTG group.

The cardiac rhythms observed at the time of CA and at the end of the 4th min of untreated asphyxia CA are shown in Table 2. CPP initially increased from baseline in all groups after the onset of asphyxia, with a peak between the 3th and the 4th minute. After this time-frame, CPP and MAP declined rapidly in all groups.

3.3 CPR Period

During CPR, MAP was maintained in all groups and at the end of the first cycle of resuscitation, but MAP remained higher in the VP (p= .021) and VP+NTG group (p= .05) at the 5th minute of CPR (E: 34.83±8.75 mmHg, VP: 66.10±28.66 mmHg, VP-NTG: 66.11±32.73 mmHg), as shown in Fig. 2.

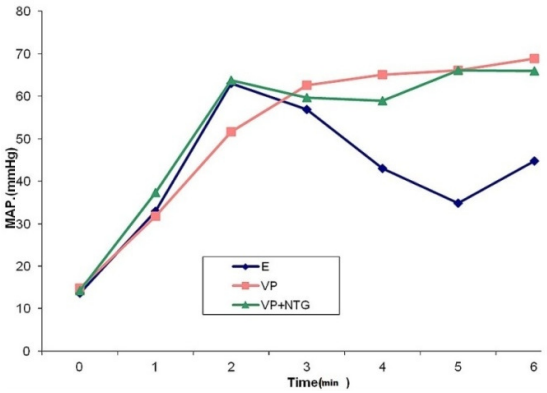

Fig. 2. Changes in mean aortic pressure (MAP) during the 6 min-resuscitation phase

Table 1. Baseline variables in the three groups

Group	E	VP	VP+NTG	p-value
Heart Rate (bpm)	119.50±21.44	123.70±5.33	119.20±13.36	NS
SAP (mmHg)	102.60±10.06	91.00±11.88	94.00±12.75	NS
DAP (mmHg)	71.10±5,45	65.00±8,83	70.40±14.77	NS
MAP (mmHg)	81.30±5.56	73.30±9.33	77.20±16.71	NS
CPP (mmHg)	62.00±5.08	57.30±8.26	63.90±14.16	NS
pH arterial	7.43±0.06	7.41±0.06	7.40±0.03	NS
$PaCO_2$ (mmHg)	35.54±5.23	39.60±4.03	41.00±2.54	NS
PaO_2 (mmHg)	134.80±13.62	128.50±18.76	138.30±11.87	NS
Weight (Kg)	20.19±1.55	19.82±0.97	21.08±2.00	NS
RADP (mmHg)	7.80±1.23	7.70±1.34	7.20±0.79	NS
RASP (mmHg)	12.00±0.82	13.20±0.92	12.40±1.96	NS

All values are presented as mean±SD; bpm: Beats per minute; RADP: Right atrial diastolic pressure; RASP: Right atrial systolic pressure. The remaining abbreviations are explained in the text

Table 2. Cardiac rhythm: Pre-asphyxia; prior to endotracheal tube clamping, LOAP; Loss of aortic pulsation, Pre-CPR; Immediately prior to CPR. NSR: Normal sinus rhythm

	E	VP	VP+NTG
Pre asphyxia	NSR (10)	NSR (10)	NSR (10)
LOAP	PEA (6)	PEA (8)	PEA (9)
	Asystole (2)	VF (2)	VF (1)
	VF (2)		
Pre CPR	VF (8)	VF (6)	PEA (5)
	Asystole (2)	PEA (2)	VF (5)
		Asystole (2)	

The maximal value of MAP during CPR in the E group was 79.67±14.18 mmHg in survivors and 40.25±3.3 mmHg in the animals that subsequently died (p< .0005). The corresponding values were 106.25±0.96 mmHg versus 66.50+23.89 mmHg (p< .012) in the VP group, and 118.33±32.24 mmHg versus 61.14±30.87 mmHg (p= .029) in the VP+NTG group.

Coronary perfusion pressure (CPP) response during resuscitation can be seen in Fig. 3. CPP was maintained in all groups during the first 3 minutes of resuscitation, but it was lower in the E group at the 5th min of CPR, when compared to the VP group (p=.029), or to the VP+NTG group (p= .025). Respective CPP_{5min} values were 5.33±4.41 mmHg, 21.10±16.11 mmHg and 23.44±29.33 mmHg.

Maximal CPP during CPR in the E group was 54.67±8.76 mmHg in animals with ROSC and 15.75±3.3 mmHg (p< .0005) in animals without ROSC. Respective values were 65.0±2.45 versus 25.83±15.9 mmHg (p= .002) in the VP group, and 78.33±4.35 mmHg versus 27.29±21.68 mmHg (p= .02) in the VP+NTG group.

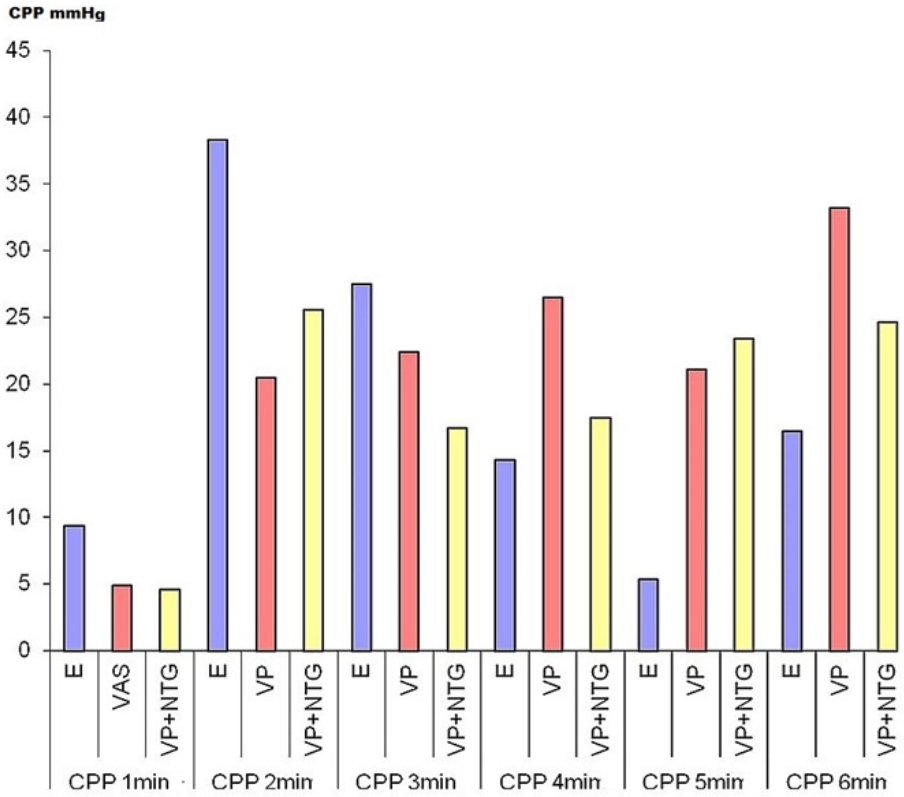

Fig. 3. Coronary perfusion pressure. Progression of coronary perfusion pressure during CPR

Statistically significant difference in $PETCO_2$ during CPR was seen only at the 2nd min of CPR between E and either the VP-NTG or the VP group. $PETCO_2$ fluctuations are shown in Fig. 4. Final $PETCO_2$ achieved in animals with ROSC during CPR in the E group was 34.67±11.88 mmHg and 10.50±7.19 mmHg (p= .007) in animals without ROSC. Correspondingly, final $PETCO_2$ was 19.25±2.87 mmHg versus 11.17±8.66 mmHg (p= .115) in the VP group, and 32.33±8.386 mmHg versus 11.71±5.648 mmHg (p= .002) in the VP-NTG group.

3.4 Post-CPR Period

In the E group, six of ten piglets were successfully resuscitated, of which two after the first cycle (no defibrillation required), two after the second cycle (one defibrillation) and two in the third cycle (two defibrillations). All six were successfully extubated and survived for 24 hours after cardiac arrest; however, severe neurologic impairment was detected in all, as described below. In the VP group, five of ten piglets were successfully resuscitated, all after the third cycle (2 defibrillations). Four of five were successfully extubated, but, in the remaining animal, VF was recorded 4 min after ROSC, but prior to extubation. Two further piglets died two and four hours later, respectively, after ROSC, due to progressive cardiorespiratory failure. ROSC was observed in three of ten animals in the VP-NTG group. More specifically, one animal was successfully resuscitated without defibrillation and two after two defibrillations; they were successfully extubated, but one died 45 min after ROSC (Table 5).

No internal organ damage or rib cage fractures were detected in any animal. Histology revealed lung fibrosis in one animal in the VP-group and in one in VP-NTG-group. During the entire post-resuscitation period, no statistically significant differences were found between the three groups, with respect to the following variables: HR, $PETCO_2$, SpO2 and PCO_2. By contrast, significant differences were present from the 10th until the 30th min in CPP and MAP, as seen in Table 3.

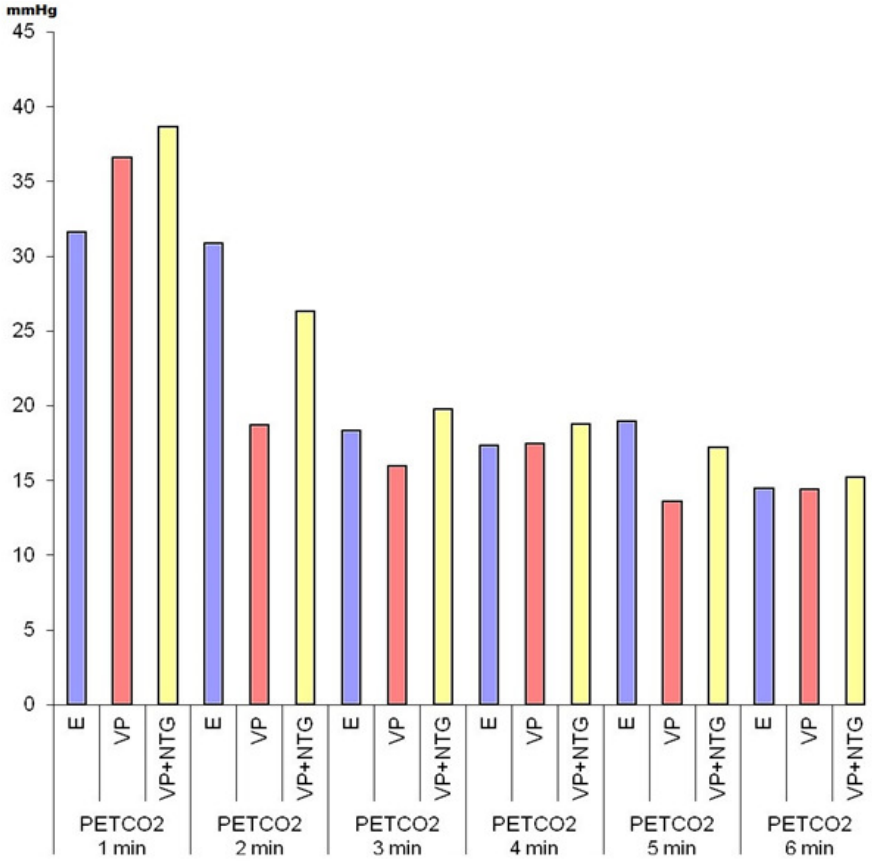

Fig. 4. Changes $PETCO_2$ during CPR

Table 3. Variable 30 min after ROSC

	E	VP	VP+NTG	p
HR	145.00±29.88	132.25±25.12	145.67±32.62	NS
SpO2	95.1±1.31	94.6±1.24	94.42±1.62	NS
PCO_2	48.67±11.48	49.50±2.38	62.67±24.54	NS
$TECO_2$	34.17±11.25	41.50±10.41	43.67±3.79	NS
MAP	67.83±13.61*	57.50±5.80*	38.33±6.66	0.011
CPP	50.67±12.93*	43.00±6.38*	24.00±7.81	0.023

** p<0, 05 vs VP+NTG; All values are presented as mean±SD*

3.5 Neurologic Outcome

Neurologic alertness was higher in the VP and VP-NTG groups, compared to the E group (Table 5). Neurologic evaluation 24 hours after ROSC revealed a NDS of 100 in all survivors of the groups of VP (100,100) and VP-NTG (100,100). This score was higher (p= .024) than the NDS of 43±5.2, calculated for the survivors in the E group, in which the following scores were assigned: 50, 50, 40, 40, 40, 40; neurologic dysfunction was prominent in the E group, with the animals appearing disoriented, responding only to painful stimuli and making unsuccessful attempts to stand (Fig. 5). To account for differences in mortality, the NDS was recalculated for all animals with ROSC, irrespective of the final outcome, as previously [19]; however, such analysis failed to reveal significant differences between groups.

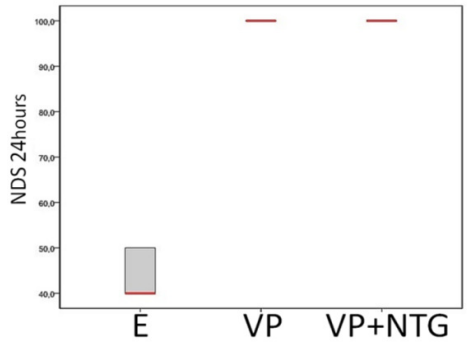

Fig. 5. NDS. Box plots of NDS in groups. The shaded area indicates the standard deviation and the horizontal lines above and below the shaded area represent the maximal and minimal values, respectively

Brain morphology after 24 h in E group displayed a mean HDS of 18.17±2.4; in more detail, the following scores were assigned: 19, 15, 14, 10, 19, 14. This contrasted the HDS in the VP group, which was improved (p= .001) at 6 ± 0 (both survivors had a score of 6), whereas the group VP-NTG showed a brain HDS of 10 ± 0 (Table 4). In all groups, mild perivascular edema was observed, but capillary congestion was rare, and brain cell necrosis was absent. Injured neurons were seen adjacent to neurons with normal appearance, mostly located in the frontal and temporal cortex. Overall, the amount of neuronal damage was high in E group, mild in VP-NTG group and low in VP group (Figs. 6, 7, 8). The average total HDS correlated well with the NDS (r= -0.753).

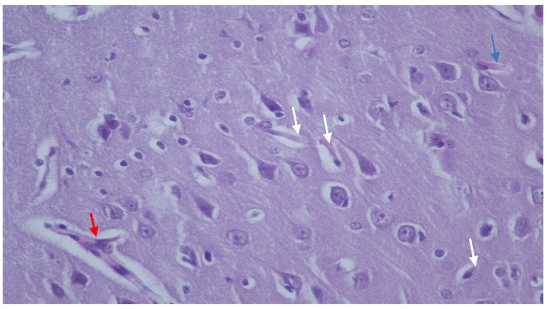

Fig. 6. Frontal cortex, group E. Representative histologic image of the frontal cortex from an animal in the group E. Multiple ischemic neurons display eosinophilic cytoplasm and pyknotic nuclei (representative neuron marked with white arrows). Capillary congestion (red arrow) and edema are also evident (magnification ×400)

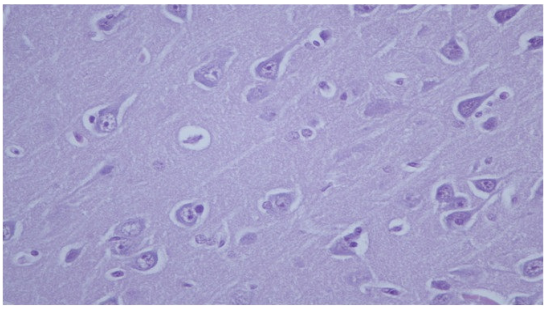

Fig. 7. Frontal cortex, group VP. Representative histologic image of the frontal cortex from an animal in the group VP. Note the normal appearance (magnification ×400)

Table 4. The results of HDS between the groups

Group	E (mean±SD) n=6	VP (mean±SD) n=2	VP+NTG (mean±SD) n=2	p-value
HDS	18.17±2.4	6.0±0.0**	10.0±0.0*	0.001

** p<0.05 vs E , ** p<0.005 vs E*

Table 5. Results between the groups in comparison

Group	E	VP	VP-NTG	p-value
CA inducing time (p-value=NS)	9.18±1.68 min	8.97± 1.42 min	8.8 ±1.88 min	NS
Total shocks	6	10	4	
ROSC (p-value=NS)	6	5	3	NS
24 hr survival	6	2	2	.091
24 hr NDS	43±5.2	100±0	100±0	.024

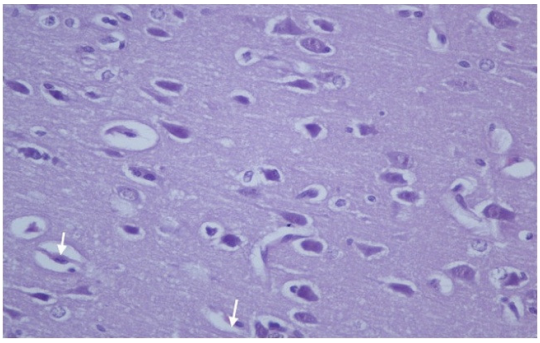

Fig. 8. Frontal cortex, group VP-NTG. Representative histologic image of the frontal cortex of group VP-NTG. Fewer condensed neurons (white arrows) and mild edema are evident (magnification ×400)

4. DISCUSSION

4.1 Experimental Animal Model

Asphyxia is the most common cause of cardiac arrest in children [1], often leading to hypoxemic encephalopathy [4]. In our experimental model of asphyxia, the observed rhythm disturbances included asystole, pulseless electrical activity and VF, in accordance with findings in pediatric populations [23]. Progressive hypercapnia after endotracheal tube clamping was seen in all groups, followed by progressive loss of aortic pulsation, in accord with previous observations in a canine-model [24]. Moreover, the duration of asphyxia time interval (~12 min) represents the average delay for the arrival of emergency medical personnel and the initiation of CPR [2], albeit longer periods are occasionally encountered. Based on these characteristics, the pediatric porcine model utilized in the present study is of considerable value in the study of asphyxia CA in children.

4.2 Neurological Outcome

The present study shows that the use of vasopressin, or vasopressin combined with nitroglycerin, improves early neurological outcome, when compared to epinephrine. Specifically, our data show reduced neurological deficits and histopathological confirmation after VP-treatment, with ameliorated brain edema and ischemic cell damage, 24 h after ROSC. However, this was counterbalanced with absence of survival benefit, which, in fact, tended to be lower in VP-treated animals; furthermore, the gain in neurological outcome was diluted, when the animals with ROSC were analyzed. Hence, the observed survival-differences, resulting in small number of observations in the VP group, impose a significant confounding factor; thus the higher NDS in this group should be viewed only as trend, requiring further validation in future studies.

4.3 Vasopressin in CA

Cerebral perfusion, determined by MAP [25], is the most important parameter affecting the extent of brain injury. In our study, MAP was maintained in the VP and VP+NTG groups at the early CPR-period, providing an explanation for our findings. To this end, the vasoconstrictive effects of VP in the skin, skeletal muscles and intestine, may divert blood to the brain, thereby maintaining adequate cerebral blood flow during resuscitation [26,27].

Vasopressin, displaying longer half-life (10-20 min) than epinephrine (4 min) [28], is being currently evaluated as a potential alternative to epinephrine in resuscitation algorithms. The rationale is based on earlier studies, reporting higher concentrations of endogenous vasopressin in patients who were successfully

resuscitated than in those who died [29,30]. Other studies have reported that increased plasma ACTH and cortisol concentrations, induced by VP, may maintain hemodynamic stability and improve ROSC rates [31]. Recently, in a rat-model of asphyxial CA, it was suggested that VP, alone or combined with E, may prevent the activation of mitogen-activated protein kinase and c-Jun N-terminal kinase signaling pathways and reduce neuronal apoptosis during CPR [32]. These salutary effects of VP may provide additional explanation for the improved neurologic outcome in our animal-cohort.

A worrisome finding in our experiments was the lower MAP in the VP and VP+NTG groups at later stages that may account for the equivocal findings in these groups, regarding survival and neurologic outcome. Thus, our findings indicate that prolonged vasoconstriction, induced by the longer half-life of VP may impair ROSC and survival. The increased afterload, caused by prolonged vasoconstriction, increases myocardial oxygen demand, leading to transient hypoxemia, impaired microcirculation, myocardial dysfunction and lethal rhythm disturbances [10]. This inference is supported by the time of death in the VP group, in which two animals died from progressive heart failure, two and four hours after ROSC.

4.4 Coronary Perfusion Pressure

CPP correlates directly with myocardial blood flow, and is considered a reliable predictor of successful CPR [32]. Improved short-term survival rates were linked to CPP above 30 mmHg generated by CPR, along with adequate end–tidal CO_2 [33,34]. Thus, CPP has been established as a highly predictive indicator of the likelihood of ROSC, validated in animal and human studies [32-34]. Such conclusions were reiterated in our experiments, in which successfully resuscitated animals achieved satisfactory CPP-values, irrespective of treatment-allocation.

CPP was maintained during CPR in our VP-treated animals, but this was only of brief duration. Specifically, CPP was higher in the VP and VP+NTG groups at the 5^{th} minute of resuscitation, but lower than in the E group at the 30^{th} minute. We feel that this finding can largely explain the lack of survival benefit after VP or VP+NTG, although our study was underpowered to detect differences in mortality. This observation may also reflect the relatively low dosage of vasopressin, used in our protocol. Indeed, a dose response-study in pigs indicated that optimal results may be expected with vasopressin dosage as high as 0.8U/Kg [35]. However, these results need to be further validated, as potential benefits may be counterbalanced by the impairment of myocardial blood flow [13,36], as discussed above. Along these lines, the wide variation in VP-dosages may provide an explanation for the results of three randomized controlled trials [15,37,38] and a subsequent meta-analysis [39], reporting similar survival with vasopressin versus epinephrine as a first-line vasodepressor agent in CA.

4.5 Epinephrine in CA

Epinephrine, currently the preferred vasopressor agent during CPR, causes systemic vasoconstriction; epinephrine activates both α1- and β1-adrenergic receptors, with vasomotor responses displaying dose-dependent curves. At high doses, as in those used in current algorithms and in the present work, the α1-vasoconstrictive effects prevail [9,27]. Thus, decreased cerebral blood flow, secondary to excessive vasoconstriction, may account for the poorer neurological outcome observed after E in our experiments.

4.6 Nitroglycerin

Theoretically, a combination of vasodepressor and vasodilator agents may exert beneficial effects during CPR, in terms of enhancing myocardial [16] and cerebral blood flow [40,41], thereby improving short-term survival. Specifically, VP in combination with NTG has been shown to increase survival rates in a 6min-asphyxia rat-model [17]. In keeping with these results, we report maintained CPP and MAP levels during CPR after VP+NTG, but these were not translated into survival benefit, as in the case of VP. NTG, a nitric oxide donor [42], causes arterial and venous vasodilation by vascular smooth muscle relaxation [16]; furthermore, NTG increases blood flow to vital organs [16], and decreases myocardial oxygen demand due to the preload reduction [43]. Despite these salutary effects, the actions of NTG on cerebral blood flow remain ambiguous [42]. In our study, the addition of nitroglycerin to vasopressin was associated with favorable neurologic outcome, when compared to the E group, but similar to that observed with vasopressin alone. Thus, at the dosages used here, the vasoconstrictive actions

of VP seem to prevail; dose-response studies are deemed necessary for more accurate assessment of NTG.

In contrast to the early benefit, a decrease in CPP and MAP was noted in the VP+NTG group, between the 10th and the 30th min after ROSC. Despite the short half-life of NTG in the setting of normal cardiac output, this finding may be attributed to the delayed vasodilating actions of NTG in the post-resuscitation phase. Our results caution the use of NTG after asphyxial CA, and call for further research on the actions of NTG on the cerebral and myocardial circulation during the early and delayed post-resuscitation phases.

4.7 Strengths and Limitations

We feel that this study adds important information on the resuscitation strategies after asphyxial cardiac arrest in pediatric populations. The large animal model, opted in our experiments, displays close resemblance with human pathophysiology, enabling clinically pertinent conclusions. We focused on the neurological outcome, given the high incidence of hypoxemic encephalopathy after asphyxial CA. Despite these merits, four limitations of the present study should be acknowledged. *First*, as we did not perform dose-response experiments, we cannot comment as to whether different vasopressin or nitroglycerin regimes (e.g. delayed administration, or repeated dosages, as previously advocated [16]) would have elicited different hemodynamic results. *Second*, we did not examine the combination of E and VP, as other studies suggested [43]. *Third*, we did not use hypothermia during the resuscitation period, despite its neuroprotective actions, according to animal [19] and human [44]. data. *Fourth*, because of our small sample size, the statistical power for survival analysis is low.

5. CONCLUSION

In experimental asphyxial CA, vasopressin, either alone or with the addition of nitroglycerin, reduced neurological deficits and histopathological damage 24 hours after ROSC, when compared to epinephrine. Further studies are required, examining the effects of these agents on ROSC and survival; these should incorporate several end-points, such as coronary perfusion pressure, left and right ventricular function, as well as vasoactive responses in the systemic and pulmonary circulation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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