Establishing An Asphyxial Pulseless Electrical Activity Arrest Model In Rabbits

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Abstract

Objective: Pulseless Electrical Activity (PEA) is an increasingly frequent cardiac rhythm in the arrested patient. We determined to establish an intact animal model of asphyxial PEA of variable duration that may be utilized for evaluation of agents of potential benefit in this scenario.

Method: Instrumented adult New Zealand White rabbits underwent induction of hypoxic PEA via tracheal cross-clamping. Resuscitation with mechanical ventilation, external chest compressions, and intravenous saline bolus was instituted following 2, 4, and 6 minutes of asphyxial arrest. Adrenaline at 100 mcg/kg was administered at 5 minutes intervals, and DC defibrillation for VT/VF. Coronary perfusion pressure (CPP), mean arterial pressure (MAP), and return of spontaneous circulation (ROSC) were recorded.

Results: Time to PEA was 279 +/- 57 seconds. No difference in CPP prior to ROSC was observed between groups. Seven of 11 (64%) animals exhibited ROSC following 2 minutes cardiac arrest, 3 of 7 (42%) following 4 minutes, and 1 of 6 (17%) following 6 minutes (p=0.065). No difference in MAP following ROSC was observed between groups.

Conclusion: We have described a reliable model of asphyxial PEA in rabbits that may be utilized for evaluation of treatments in hypoxic cardiac arrest. Practical suggestions for successful adoption are given.

Introduction

The incidence of pulseless electrical activity (PEA) as a presenting cardiac rhythm in community cardiac arrest appears to be increasing commensurate with an observed reduction in the incidence of ventricular fibrillation [1,2]. Whilst the origins of this change may be debated [2,3], renewed attention has focussed on the treatment of PEA and asystole in cardiac arrest. The aetiology of PEA in cardiac arrest is far from uniform. Multiple pathophysiological processes may ultimately result in a state of cardiac electrical activity without discernable mechanical output [4]. The magnitude of cardiac dysfunction in PEA may additionally vary from absent cardiac wall motion (true PEA), to states of clinically undetectable blood pressure but with residual cardiac activity (pseudo-PEA) [5]. The development of asphyxial PEA furthermore differs significantly from that of alternate cardiac arrest models (induced ventricular tachycardia/fibrillation) in that the progression to zero cardiac output is more gradual, and associated with far greater accumulated metabolic derangement and oxygen debt in the former, as opposed to abrupt in the latter.

Numerous previous investigators have validated animal models of cardiac arrest in both large [6-8], and small [9-11] animal species. Few however have been specifically developed for evaluating treatments for PEA. The purpose of the present study therefore is to develop and document the establishment of a rabbit model of asphyxial PEA, with varying duration of precipitating hypoxic insult. It is expected that this model would prove useful for further laboratory research directed at the treatment of this increasingly important clinical entity.

Methods

The experiment was performed at the Ruakura Animal Research Facility, Hamilton, New Zealand. All study protocols were approved by the Ruakura Animal Ethics Committee. Animals were managed in accordance with institutional guidelines for ethical animal experimentation.

Model Preparation

Adult New Zealand White rabbits of mixed gender were studied. Enclosures were gender specific with no chance of pregnancy. Free access to feed and water, and standard 12-hour reverse day/night cycle were maintained prior to utilisation. Ten minutes prior to surgery animals were sedated with ketamine at 50mg/kg and xylazine at 4 mg/kg via intramuscular injection. Animals were placed supine on a warming board maintained at 37 degrees Celsius before undergoing venous cannulation of the marginal vein of the ear. Additional 1-mL bolus’s of anesthetic solution (ketamine 10 mg/mL and xylazine 2 mg/mL) were administered on evidence of animal distress.
during subsequent invasive procedures. Following subcutaneous instillation of 1 mL 1% lignocaine a transverse incision was made in the base of the neck. Blunt dissection was used to expose the trachea, left common carotid artery, and confluence of the internal jugular vein and superior vena cava on the right. Tracheostomy was performed with 3.5mm internal diameter endotracheal tube inserted 10mm caudal to the larynx during spontaneous respiration. This was advanced 15mm into the trachea and secured by taping. One saline filled 18-guage polyethylene catheter was advanced from the carotid artery into the thoracic aorta for measurement of aortic pressure. This was connected in standard fashion (Edwards Lifesciences pressure transducer, Irvine, CA) to Hewlett-Packard 78834A neonatal monitor. Another 18-guage polyethylene catheter was advanced through the vena cava into the right atrium and identically connected to the monitoring system. Both pressure transducers were room air zero-calibrated with reference to the mid-chest. Continuous three-lead electrocardiogram with subcutaneous electrodes was instituted, with standard lead 2 monitored for the duration of the experiment.

Induction PEA and Resuscitation Protocol
Following a ten-minute equilibration period after completion of invasive procedures, during which time animals breathed room air, asphyxial cardiac arrest was induced by tracheal cross-clamping. Cardiac arrest was determined by a MAP of less than or equal to 20 mmHg, with associated pulse pressure of 1mmHg or less. Animals were randomly assigned to one of three groups according to duration of induced PEA arrest. Groups included PEA arrest time of two, four, and six minutes total duration.

Following the determined period of asphyxial cardiac arrest, the endotracheal tube was unclamped and basic life support (BLS) CPR commenced. Ventilation was via Nuffield series 200 pediatric ventilator (Penlon Ltd, Abington, England) and provided 50 breaths/minute 100% oxygen at 0.25 L/sec with inspiration: expiration ratio set at 1:2 in the absence of positive end expiratory pressure. Mechanical chest compressions were delivered by custom manufactured device providing 180 compressions per minute with 1:1 compression/relaxation ratio. Compression depth was adjusted to approximately 30% chest diameter. Chest compressions were interrupted for 10 seconds every minute to assess native hemodynamic metrics. After two minutes of BLS CPR animals received 3 ml/kg 0.9% saline solution by intravenous injection over a two-minute period. Saline bolus was elected to provide control comparison for future experimental agents in this arrest model. Advanced cardiac life support (ACLS) interventions were commenced four minutes following initiation of resuscitation. Adrenaline was administered at dose 100 mcg/kg [diluted to 3mL], at four minutes and repeated at five minute intervals in animals failing to display ROSC. External electrical defibrillation [20J monophasic DC via saline soaked paddles placed at the base of the neck, and inguinal region] was additionally administered for ventricular tachycardia/fibrillation. Advanced cardiac life support interventions were continued until return of spontaneous circulation, or discontinuation of study protocol. Return of spontaneous circulation (ROSC) was defined as an unassisted pulse with systolic arterial pressure of 50 mmHg or higher for 3 minutes or longer. Resuscitative efforts were discontinued 14 minutes after cardiac arrest in animals failing to develop ROSC. All animals that exhibited ROSC were monitored to 50 minutes with acquisition of hemodynamic parameters at one-minute intervals. CPR was not re-commenced following initial ROSC in animals subsequently developing a second period of cardiac arrest.

Acquisition of hemodynamic metrics
Heart rate and mean arterial pressure (MAP) were transcribed directly from the monitoring system to standardized data collection template. Coronary perfusion pressure (CPP) was calculated as the difference between minimal diastolic aortic and simultaneously recorded right atrial pressure. Heart rate was recorded to the nearest beat per minute. Pressure metrics were recorded to the nearest mmHg. At termination of the study protocol all surviving animals were killed with pentobarbitone overdose (900mg via rapid intravenous bolus). Necropsy was performed after death to confirm positioning of endotracheal tube, and vascular catheters.

Statistical Analysis
Statistical analysis of all variables was performed with SPSS for Windows (version 10.0, SPSS, Chicago, IL). The distribution of quantitative variables was examined to detect significant departure from normality by the Kolmogorov-Smirnov test. Kruskal Wallis and Two-way analysis of variance (ANOVA) were used as appropriate to determine statistical significance between continuous variables (presented as mean +/- SD). Fishers exact testing was used to determine statistical significance in dichotomous outcomes. The critical P value retained for significance was 0.05.

Results

Twenty-four rabbits were used for the experimental
protocol. Animal characteristics and baseline hemodynamic parameters are presented in table 1. All metrics were interrogated with, and passed the Kolmogorov-Smirnov test for normality. No statistically significant difference in any baseline parameter between groups was observed. Time from tracheal cross-clamping to onset of PEA was 279 +/- 57 seconds. Mean arterial pressure during PEA induction is presented graphically in illustration 1.

**Data presented as Mean (SD)**
Cardiac arrest rhythm at PEA in the two-minute group was: 2:1 atrioventricular block in 3; sinus tachycardia in 3; sinus bradycardia in 3; complete heart block in 2. Cardiac arrest rhythm at PEA in the four-minute group was: complete heart block in 2; 2:1 atrioventricular blockade in 2; sinus bradycardia in 2; sinus tachycardia in 1. Cardiac arrest rhythm in the six-minute groups was: complete heart block in 4; 2:1 atrioventricular block in 1; sinus bradycardia in 1. Seven animals (64%) in the two-minute group, 3 animals (42%) in the four-minute group, and one (17%) in the six-minute group exhibited return of spontaneous circulation (p=0.068). Time to ROSC was 494 +/- 133sec, 700 +/- 125sec, and 660 sec in the 2, 4, and 6 minute groups respectively. No animal exhibited ventricular tachycardia, or ventricular fibrillation during the course of the experiment.

Generated coronary perfusion pressure prior to ROSC for all groups is displayed graphically in illustration 2. Mean arterial pressure during PEA induction is presented graphically in illustration 3. Spontaneous circulation at 50 minutes was maintained in four of seven animals in the two-minute PEA group (57%), one animal in the four-minute PEA group (33%), and no animals in the six-minute PEA group.

**Outcome of necropsy**
Correct placement of vascular catheters and endotracheal tube was confirmed in all animals. No adverse events associated with invasive procedures or other traumatic injuries were found.

**Discussion**

In the present study we have demonstrated reliable production of pulseless electrical activity following graded asphyxia in whole rabbits. Return of spontaneous circulation was demonstrated to correlate inversely with duration of initiating hypoxic insult as was eventual resuscitation outcome. This model may be gainfully employed to design and power future studies examining response of experimental agents and procedures in resuscitation from asphyxial PEA. The rabbit is an uncommonly utilised animal in investigations of experimental cardiac arrest. This species however confers a number of advantages compared with more commonly employed murine, canine, or porcine models. Rabbits are comparatively less expensive than alternative larger animals, yet adults are of a size enabling ready instrumentation and subsequent monitoring of conventionally recorded invasive pressure metrics. Rapid growth form juvenile to adult further enhances the attraction as a species. A number of features unique to the present experimental model and utilized species warrant additional comment. Sedation during the present experiment was achieved with Ketamine and Xylazine administered via intramuscular and intravenous routes. One obvious advantage in this approach is the absence of volatile anaesthetic agents and the inherent requirement for gas storage, administration, and scavenging required when inhalational anaesthesia is utilised. This anaesthetic combination was furthermore adopted following reports of relative cardiovascular stability in rabbits [12,13] and provided excellent surgical anaesthesia in the current model.

Muscle relaxants were not administered in the present study prior to tracheal cross-clamping. This gives the animal an opportunity to gasp and produces a state of stress following airway occlusion. Endogenous catecholamine discharge during this period is likely to be maximal in an effort to maintain cardiovascular homeostasis prior to cardiac arrest. The metabolic consequences of combination hypoxia, skeletal muscle contraction, and endogenous catecholamine secretion are however likely heterogeneous, and may have contributed to variability in pH, systemic oxygen debt, and lactacidemia at cardiac arrest. All may potentially contribute to eventual resuscitation outcome [16]. We are unable to comment on any such variability in the present experiment given the absence of metabolic metrics (arterial blood gas analysis, serum lactate estimation) at cardiac arrest. Electing to withhold muscle relaxants was however adopted in line with clinically relevant arrest scenarios. In the present study ROSC in all animals followed the administration of at least one dose of adrenaline. No animal exhibited ROSC during BLS resuscitation alone. This finding is in line with previous reports iterating the exquisite sensitivity of murine and ovine [17,18] models to catecholamines. Significant inter-species variability in adrenaline responsivity is however known to exist. As such generalisation of the present findings to alternate animal models, must proceed with significant caution. Furthermore, adrenaline at high dose is known to be associated with severe post resuscitation myocardial dysfunction, and late
deterioration in cardiovascular performance [14,15]. The low survival to 60 minutes in animals exhibiting initial ROSC in the present model may be in part be attributable to such a phenomena. Ketamine, utilized as sedation, may have additionally contributed to global adrenergic tone via its recognized sympathomimetic action. A number of limitations are inherent in the present study. Uneven numbers of animals were utilised in each arm of PEA induction. As such ROSC and survival to 50 minute data comparison presents some difficulty. This observation is further compounded by the lower than expected 50 minute survival rates for animals attaining ROSC. Key study parameters in the present study were collated by un-blinded investigators. As such potential for observer, or information, bias exists. Metrics were however transcribed directly from monitoring equipment in systematic fashion. Finally, the study was by nature largely descriptive. Given the absence of key metabolic parameter estimation, we are unable to comment on severity of initial metabolic insult, or the contribution of such to outcome. A number of practical suggestions are offered to enhance utilisation of the described model. These include: 1) A minimal incision (1.5cm) can be used to expose the tracheal, internal carotid artery, and superior vena cava with sufficient blunt dissection. 2) Vascular cannulation of the internal carotid and superior vena cava are readily performed with standard percutaneous catheter-over-needle devices. 3) Distal stabilization of the internal carotid artery and vena cave with a forcep or ligature increases successful cannulation. 4) Aspiration, and subsequent filling vascular cannulae is essential to avoid air embolisation. 5) Securing of vascular catheters can be achieved with an ordinary rubber band rather than ligature. 6) The right atrial catheter must be advanced slowly, and withdrawn immediately on evidence of arrhythmia indicitative of atrial irritation. 7) External chest compressions are most efficiently delivered with the plunger in slight left parasternal location.

Conclusion(s)

The described rabbit model of asphyxia is able to reliably generate PEA from which resuscitation with standard advanced cardiac life support measures correlates inversely with duration of hypoxia. Our experience indicates this to be a simple, convenient, and economical alternative to larger animal species. This model may be gainfully employed in future studies exploring potential treatments for hypoxic PEA.

Abbreviation(s)

PEA: pulseless electrical activity
DC: direct current
VT: ventricular tachycardia
VF: ventricular fibrillation
CPP: coronary perfusion pressure
MAP: mean arterial pressure
ROSC: return of spontaneous circulation
CPR: cardiopulmonary resuscitation
ANOVA: analysis of variance

References

Illustrations

Illustration 1

Mean Arterial Pressure (MAP) during induction Pulseless Electrical Activity.

Illustration 2

Coronary Perfusion Pressure (CPP) during resuscitation.
Illustration 3

Mean Arterial Pressure (MAP) in animals exhibiting return of spontaneous circulation. n=7 (2 min group), n=3 (4 min group), n=1 (6 min group).
Illustration 4

Table 1: Animal characteristics and baseline hemodynamic metrics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2 min (n=11)</th>
<th>4 min (n=7)</th>
<th>6 min (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>89.5 (5.2)</td>
<td>89.5 (6.8)</td>
<td>84.2 (1.2)</td>
<td>0.196</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>6:5</td>
<td>2:5</td>
<td>2:4</td>
<td>0.507</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2426 (183)</td>
<td>2284 (353)</td>
<td>2220 (205)</td>
<td>0.154</td>
</tr>
<tr>
<td>Heart rate (BPM)</td>
<td>213 (30)</td>
<td>179 (19)</td>
<td>221 (22)</td>
<td>0.150</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>85.9 (7.0)</td>
<td>89.7 (11.8)</td>
<td>88.3 (5.4)</td>
<td>0.817</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70.1 (5.8)</td>
<td>70.3 (10.1)</td>
<td>74.7 (5.5)</td>
<td>0.358</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>76.7 (5.5)</td>
<td>79.5 (10.3)</td>
<td>81.2 (5.9)</td>
<td>0.300</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>6.6 (1.9)</td>
<td>8.3 (4.0)</td>
<td>5.8 (0.75)</td>
<td>0.650</td>
</tr>
<tr>
<td>CPP (mmHg)</td>
<td>63.5 (5.7)</td>
<td>62.0 (13.7)</td>
<td>68.8 (5.3)</td>
<td>0.322</td>
</tr>
</tbody>
</table>

BP: Blood Pressure
MAP: Mean Arterial Pressure
RAP: Right Atrial Pressure
CPP: Coronary Perfusion Pressure
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