DEVELOPING A NEW PHARMACOLOGICAL THERAPY USING DIFFERENT COMBINATIONS OF ADENOSINE, LIDOCAINE AND MAGNESIUM FOR ASPHYXIAL CARDIAC ARREST IN RATS

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DECLARATION ON ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research and ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the *James Cook University Policy on Experimentation Ethics Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research practice* (2001). Relevant research methodology reported in this thesis received clearance from the James Cook University Experimentation Ethics Review. Animal Ethics numbers A1540 and A1910.

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ABSTRACT

Background

Around 17 million people die from cardiac arrest worldwide every year, but no drug therapy has been shown to improve survival. Failure to adequately rescue the heart and brain leads to post-resuscitation syndrome, including coagulopathy imbalances, organ failure and death. Adenosine and lidocaine (AL) have been shown to elicit cardioprotection in cardiac surgery and haemorrhagic shock. In an effort to translate this protection to cardiopulmonary resuscitation, the effects of AL on cardiac and haemodynamic rescue were examined following asphyxial-induced cardiac arrest in the rat.

Methods

All studies employed asphyxial-induced cardiac arrest rat model. Cardiac arrest, defined as mean arterial pressure (MAP) <10 mmHg, was induced by stopping the ventilator and clamping the line for 8 minutes. Resuscitation attempt comprises of 0.5 mL intravenous drug injection, declamping ventilator line, and chest compressions (300 min^{-1}). The end points were return of spontaneous circulation (ROSC), haemodynamics during or following resuscitation, and ECG stability at different temperatures. When applicable, coagulopathy was assessed using plasmatic PT and aPTT tests and whole blood thromboelastometry. The level of statistical significance was $p<0.05$.

Experimental design

After obtaining the optimal AL dose, the first study is designed to assess the effect of adenosine (A 0.48 mg), lidocaine (L 1.0 mg), AL (0.48/1.0 mg) and saline (0.9% NaCl) on ROSC, haemodynamic rescue and ECG stability during compression phase every 5 min over 60 min. The animal's temperature was allowed to drift (34-35^{o}C). The second study examined the effect of intra arrest and post-resuscitation moderate hypothermia (28-32^{o}C) versus normothermia (36-37^{o}C) among the different treatment groups (AL, lidocaine or saline, n=8) during intermittent compression phase and following one 60 sec set of compressions. An adenosine group was not included due to high mortality in the first study. In the third study, the effect of Mg^{2+} addition was examined during post-ROSC moderate hypothermia (28-32^{o}C) with 6 group treatments (n=8): 1) saline; 2) adenosine-Mg^{2+} (AM); 3) lidocaine-Mg^{2+} (LM), 4) AL-Mg^{2+} (ALM), 5) AL, and 6) Mg^{2+}

alone (Mg). Post-arrest coagulopathy was also assessed. The fourth study investigated the effects of ALM (n=10) compared to saline (n=12) on ROSC, ECG rhythm, post-resuscitation haemodynamics, coagulopathy and neurohistological changes following intra-arrest hypothermia and two-hour active rewarming post resuscitation. Lastly, ALM (n=12) was then compared with standard-of-care epinephrine (n=12) with the same resuscitation and temperature protocol as the fourth study.

Results

In the first study, AL led to consistently higher MAP during chest compressions ($p<0.05$; 35-45 and 55 minutes) followed by lidocaine, and was lowest with adenosine and saline. Improved ECG rhythm was apparent in AL-treated rats. No groups sustained ROSC after 5-10 min resuscitation at body temperature 34-35^{o}C. Similarly, during normothermia, no rats achieved ROSC after 10 min. However, during induced hypothermia (28-32^{o}C), ROSC was achieved in 75% controls and in 100% lidocaine and AL-treated rats. After 40 min, 37.5% of AL hypothermic rats achieved ROSC compared to 12.5% of lidocaine and none of saline rats *(X^2=56.058 df (5) $p<0.01$).* Arterial pressures were significantly higher in AL and lidocaine hypothermic rats than hypothermic controls or any normothermic groups ($p<0.05$ at 30-60 min). Saline controls (normothermic or hypothermic) experienced a large number of ventricular tachycardia (VT) and fibrillation (VF), whereas both AL groups had no VF over 60 min. With a single set of 60 sec compressions and induced hypothermia, 75% saline, 87.5% lidocaine and 100% AL-treated rats immediately achieved and maintained ROSC over the 60 min observation period. AL with Mg^{2+} (ALM) and AL only led to 100% ROSC without VF during two-hour post-ROSC hypothermia (28-32^{o}C). Two out of eight animals in saline, three in Mg, four in AM and one in LM groups did not attain ROSC. Furthermore, ALM but not AL led to significantly higher arterial pressures from 30-120 min of ROSC compared to all other groups. After 90-120 min, ALM rats had MAP ranging from 70-76 mmHg; whereas, Mg rats had the lowest pressures, with MAPs ranging from 41-46 mmHg. Following intra-arrest hypothermia and post resuscitation rewarming, 100% ALM rats achieved ROSC compared to 67% controls (χ^2=3.889, $p<0.05$), and generated higher MAP from 45-120 min post ROSC than controls ($p<0.05$ at 75-120 min). After 120 min, coagulation analysis from saline controls displayed hypocoagulopathy (prolonged EXTEM/INTEM clotting time, clot formation time, prothrombin time, activated partial thromboplastin time), decreased maximal clot firmness, lowered elasticity, and lowered clot amplitudes but no change in lysis index. These coagulation abnormalities were mostly prevented by ALM, but the presence of

neurohistopathology changes was not significantly different from controls. Compared to epinephrine, ALM achieved 100% ROSC at significantly lower MAP (39 ± 3.3 mmHg vs 129 ± 7.0 mmHg) and heart rate (59 ± 5.5 bpm vs 151 ± 13.6 bpm), while three out of 12 epinephrine rats failed to achieve ROSC due to persistent VF. After 90-120 min, arterial pressures in ALM were significantly higher than epinephrine group (81 ± 2.9 mmHg compared to 62 ± 3.9 mmHg at 120 min). Similar to ALM, epinephrine treatment mostly prevented abnormal coagulation at 120 min post resuscitation.

Conclusion

A bolus of AL administered immediately prior to chest compressions resulted in higher developed arterial pressures during compressions compared to lidocaine, adenosine or saline treatment at body temperatures 34-35^{o}C. However, ROSC was not sustained within 5-10 min in any group. Moderate hypothermia during and after resuscitation significantly improved ROSC sustainability and developed pressures during intermittent compressions, however, ROSC achievement was higher in AL than any other group. Mg^{2+} addition in AL solution (ALM) further improved post-resuscitation haemodynamics compared to saline, AM, LM, AL and Mg^{2+} alone during moderate hypothermia. ALM also improved ROSC and haemodynamics during post-resuscitation rewarming following intra-arrest hypothermia, and was superior when compared to epinephrine. Death of epinephrine-treated rats was from refractory VF, while ALM prevented fatal arrhythmias during induced hypothermia or active rewarming. Acute coagulopathy was apparent after 120 min ROSC following cardiac arrest, and both ALM and epinephrine mostly prevented the abnormalities. ALM with induced hypothermia may offer clinical benefits in cardiac arrest victims to rescue the heart and improve post cardiac arrest haemodynamics and coagulation status.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEWS

1.1 Introduction

Cardiac arrest is a medical emergency characterized by unexpected cessation of breathing and unconsciousness as a result of insufficient cardiac output and systemic blood flow due to cardiovascular collapse (Ristagno*, et al.*, 2009b). The vast majority of cardiac arrest cases are fatal with a survival rate of <10% and this has not changed over the past three decades (Kramer-Johansen, 2007; Sasson*, et al.*, 2010). The primary aetiology of cardiac arrest is related to pre-existing heart disease, with the remaining 20-25% from a non-cardiac origin, which is predominantly associated with asphyxia (Engdahl*, et al.*, 2003b; Hess*, et al.*, 2007).

Asphyxia is defined as airway obstruction or inadequate ventilation which results in hypoxaemia as well as hypercarbia (Hickey & Callaway, 2005). Asphyxia occurs from respiratory blockage, suffocation, drowning, or coma with loss of airway patency; but can also originate from drug use, haemodynamic disturbance and/or autonomic dysfunction (Ornato & Peberdy, 1996). Asphyxial-induced cardiac arrest is more common in young victims (<18 years) (Engdahl*, et al.*, 2003a), and affects around 16,000 children each year in the USA alone (Tress*, et al.*, 2010). In adults, asphyxia is a predominant cause of in-hospital cardiac arrest, accounting for more than 30% of the total cases (Sandroni*, et al.*, 2004).

The initial electrocardiographic (ECG) rhythm in asphyxial cardiac arrest victims is predominantly pulseless electrical activity (PEA) or in some cases the total absence of an ECG pulse known as asystole (Engdahl*, et al.*, 2003b). Unfortunately, the initial PEA/asystole rhythm during cardiac arrest is associated with lower chance of successful return of spontaneous circulation (ROSC), 24 hr survival, and survival to hospital discharge compared to if the first rhythm was ventricular fibrillation (VF) or ventricular tachycardia (VT) (Mader*, et al.*, 2012; Meaney*, et al.*, 2010). Percentage survival of patients with a PEA/asystole (unshockable) rhythm is <5% (Sasson*, et al.*, 2010), and this dismal statistic reflects poorly on current therapies to treat the condition (Hallstrom*, et al.*, 2009; Papastylianou & Mentzelopoulos, 2012). There is growing concern that the current therapies mostly focus on VF/VT resuscitation rather than asphyxial-related deaths (Hickey & Callaway, 2005). However, it is important to emphasize that both aetiologies of cardiac arrest in humans (cardiac and non-cardiac

origin) constitute major medical unmet needs and require new strategies and treatments to improve the current survival statistics with improved neurological outcomes. A better understanding of the precipitating pathological events during asphyxial and non-asphyxial cardiac arrest is crucial to develop new treatments or therapies.

The present chapter will discuss the pathophysiology of asphyxial cardiac arrest in a number of animal models followed by the determinant of resuscitation outcomes. It is important to note that successful resuscitation (ROSC) does not always result in a good prognosis; indeed, a range of pathological disorders can occur which lead to further morbidity and death (Nolan, et al., 2008). This post-cardiac arrest syndrome will also be reviewed along with the strengths and limitations of current standard-of-care drugs to manage post-cardiac arrest complications. Lastly, the potential benefit of adenosine and lidocaine that is currently used for arresting and reanimating the heart during cardiac surgery will be summarised. The hypothesis that will be tested in this thesis is that a modification of this new drug therapy may improve resuscitation outcomes following eight minutes of asphyxial hypoxia in rats.

1.2 Pathophysiology of asphyxial-induced cardiac arrest

Asphyxial cardiac arrest occurs secondary to respiratory failure (Engdahl*, et al.*, 2003b; Hess*, et al.*, 2007). Unlike cardiac origins, the pathophysiology of asphyxial cardiac arrest is seldom discussed and less understood. Different characteristics in this type of arrest include hypoxaemia, hypercarbia and hypotension that occur prior to circulatory or cardiac arrest (Manole*, et al.*, 2009). Following respiratory failure, inadequate oxygen delivery to body tissues evokes a number of physiological responses to hypoxia, including transient hypertension (Kubasiak*, et al.*, 2002). This is followed by direct depression of the heart's pacemaker cells and increased parasympathetic discharge, resulting in bradycardia (Ornato*, et al.*, 2005). If hypoxia is prolonged during asphyxia, bradycardia progressively turns into bradyasytole, characterized by PEA/asystole, as oxygen and nutrient supply to cardiac tissues becomes more diminished (ischaemia) (Ornato & Peberdy, 1996). At this stage, mitochondrial oxidative phosphorylation of ADP to ATP is compromised leading to anaerobic glycolysis and accumulation of lactate and hydrogen ions (intracellular acidosis) (Fig. 1.1). This hypoxia-driven set of cascades contributes to cellular dysfunction (Buja, 2005). Moreover, electrolyte transport is altered provoking protease and phospholipase activation, which in turn increases physical disruption of cytoskeleton and membrane

structures, leading to depolarization of the membrane potential and intracellular calcium loading (Braun & Anderson, 2007; Luqman*, et al.*, 2007). In myocytes, the deleterious process manifests in abnormal electrical conduction and generates arrhythmias (see Fig. 1.2). Meanwhile, the Ca^{2+} loading from sarcoplasmic and mitochondrial dysfunction also leads to a decrease in myofibrillar sensitivity, resulting in cardiac mechanical dysfunction or myocardial stunning (Fletcher*, et al.*, 2008; Ornato & Peberdy, 1996). Overall, this pathological hypoxic, metabolic, ionic and electrical dysfunction results in cardiac arrest leading to complete loss of local and systemic perfusion to the vital tissues and organs (see Fig. 1.1).

Furthermore, during this period of circulatory stand-still, which results in global cell deprivation and accumulation of waste products (see Fig. 1.1), the endothelium releases a number of inflammatory, immune and coagulation mediators (Gando*, et al.*, 2000), starting as soon as five minutes from the onset of circulatory arrest (Idris*, et al.*, 2005). Unfortunately, although reperfusion is vital for survival and prevention of cell death, the reperfusion process also aggravates the ongoing multiple, hypoxia-driven cascades that started *during* arrest (Adrie*, et al.*, 2004). As a consequence, successful resuscitation is commonly proceeded with increased inflammatory reactions (Niemann*, et al.*, 2008), immune system dysfunction (Adrie*, et al.*, 2004), and marked activation of coagulation pathways (Adrie*, et al.*, 2005). Reperfusion injury is therefore claimed to be the key factor of post-arrest derangements (Basu*, et al.*, 2003), which relate inversely with cardiac arrest survival to hospital discharge (Radhakrishnan*, et al.*, 2007).

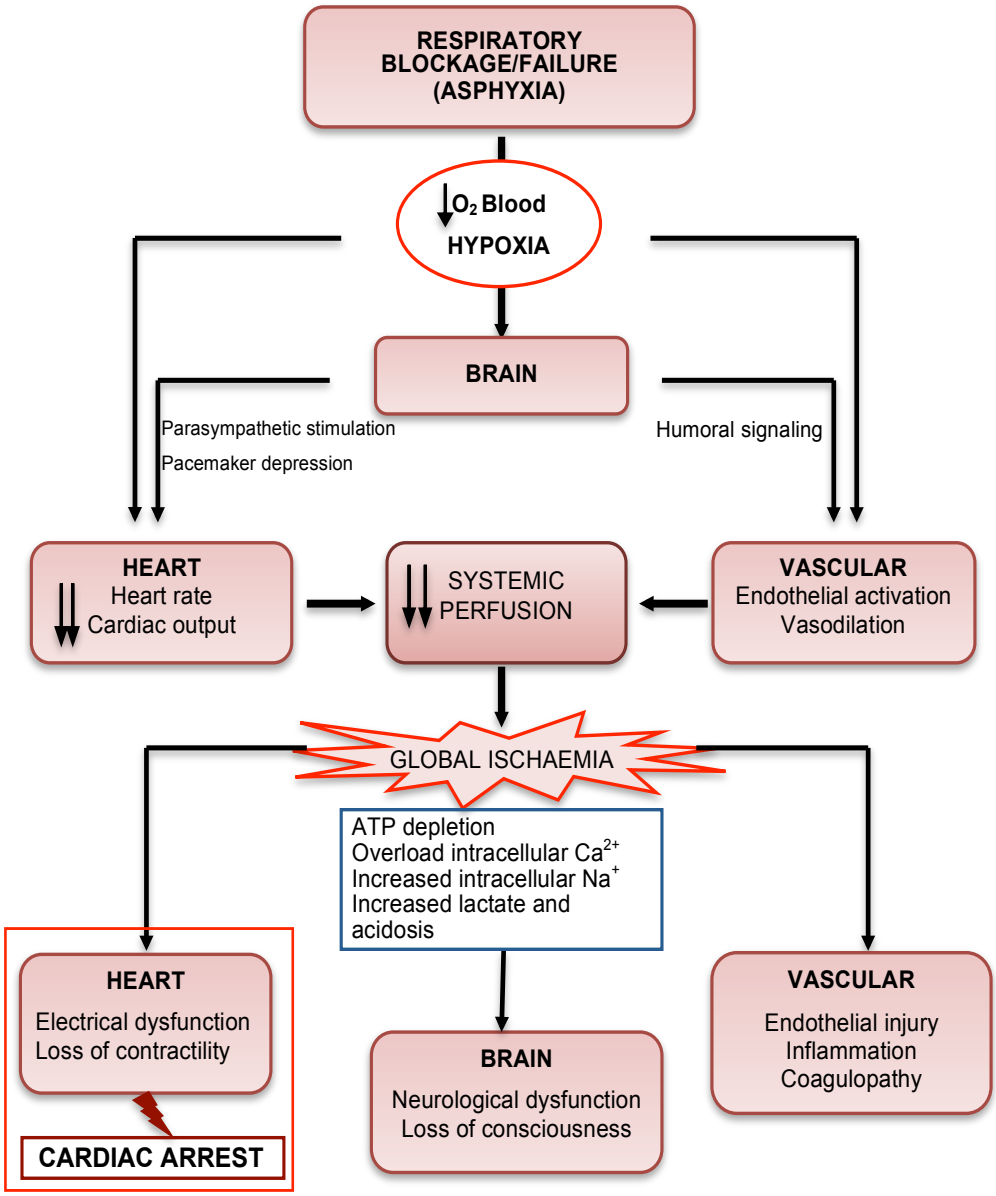


Figure 1.1 Proposed sequence of asphyxial cardiac arrest pathogenesis
Prolonged respiratory failure causes a lack of oxygenated blood and widespread tissue hypoxia. The hypoxic responses lead to bradycardia, low cardiac output and lower blood pressure, which in turn reduce systemic blood perfusion. Global hypoxia and ischaemia lead to ATP deficit, anaerobic metabolism, ionic imbalance, lactate accumulation, increased acidosis, and increased reactive oxygen species (ROS) formation. A marked increase in intracellular Ca^{2+} induces further cellular injuries and electrolyte imbalances, and increased intracellular Na^{+} leads to cell swelling. In myocardial tissue (see Fig. 1.2), these changes result in electrical and mechanical dysfunctions. Moreover, hypoxic/ischaemic pathogenesis also immediately affects brain tissues leading to loss of consciousness, and endothelial cell activation with pro-inflammatory reactions and coagulopathy.

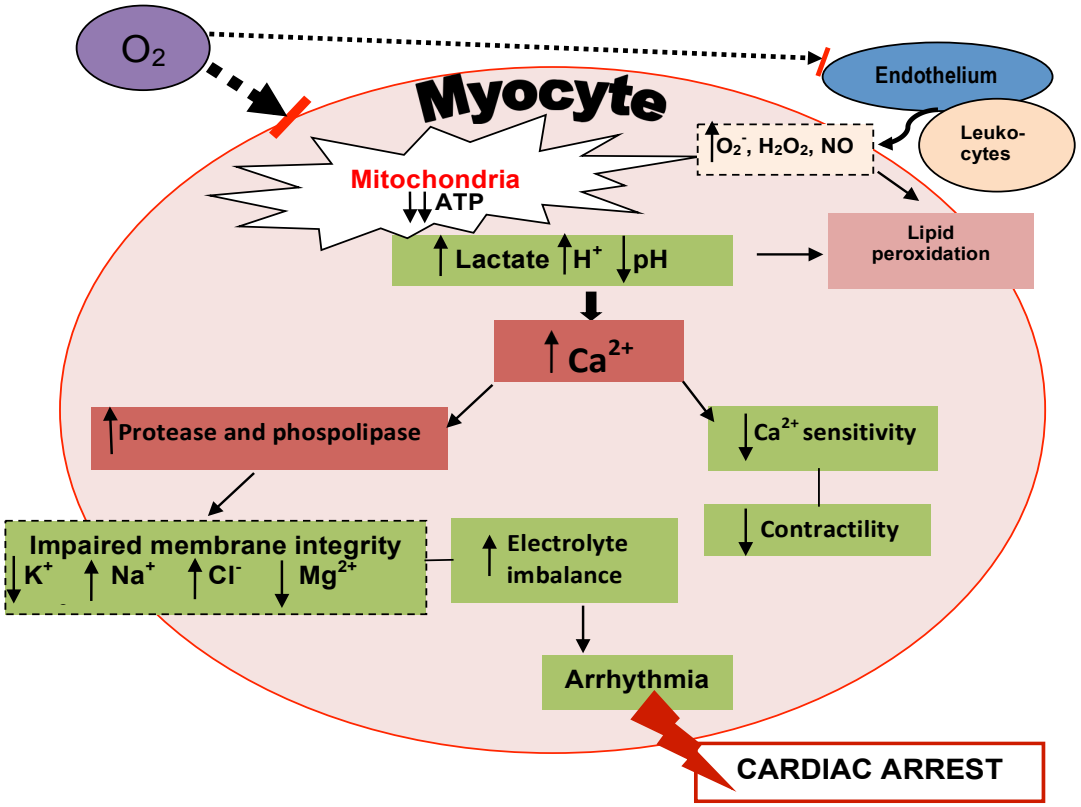


Figure 1.2 Pathogenesis of ischaemic myocyte-induced cardiac arrest
Oxygen deficiency leads to mithocondrial ATP deficit, increased lactate and acidosis, and release of reactive oxygen species (ROS). Consequently, intracellular Ca^{2+} markedly increases, which activates protease and phospolipase leading to impaired membrane permeability and subsequent electrolyte imbalance resulting in arrhythmias. Ca^{2+} overloading also induces Ca^{2+} desensitivity leading to contractility dysfunction. Persistent arrhythmias and loss of contractility result in cardiac arrest. The release of inflammatory mediators and ROS from ischaemic endothelium also contribute to cardiac arrest pathology (Adapted from Buja, 2005).

1.3 Animal models of asphyxial-induced cardiac arrest

Animal models have been used extensively in cardiac arrest research, and include rodents (Kamohara*, et al.*, 2001), canines (Vaagenes*, et al.*, 1997), swine (Ewy*, et al.*, 2010) and primates (Liang*, et al.*, 2009). Despite physiological differences, animal experiments offer investigation of more controlled variables in terms of sample variability and research designs. Notwithstanding low survival rates for over three decades, current therapeutics used to treat cardiac arrest are primarily based on animal studies. Two types of experimental models are generally employed: 1) electrical-induced model, which is believed to reflect a cardiac aetiology, and 2) asphyxial-induced cardiac arrest, which is thought to represent the non-cardiac aetiology in human cardiac arrest. The first model electrically triggers VF which leads

to cessation of systemic blood flow and a cardiac arrest state (Chen*, et al.*, 2007). In contrast, the asphyxial hypoxia model utilises obstruction of the airway and cardiac arrest arises from prolonged whole body systemic hypoxia/ischaemia (Kamohara*, et al.*, 2001).

Immediately during asphyxia, animals often demonstrate a transient increase in heart rate and blood pressure (DeBehnke*, et al.*, 1995; López-Herce*, et al.*), which then gradually switches to bradycardia and progressively turns into pulselessness (PEA and then asystole) (Hicks*, et al.*, 2000; Katz*, et al.*, 1995; Lipinski*, et al.*, 1999). The duration of apnoea required to induce cardiac arrest depends on the animal model used. In rodent models, for example, it usually takes 3-4 min (Katz*, et al.*, 1995; Lipinski*, et al.*, 1999; Xiao*, et al.*, 1998), whereas, canine and swine models may require 7-12 min of apnoea (Adams*, et al.*, 2008; DeBehnke*, et al.*, 1995; Mayr*, et al.*, 2001). Although asphyxial models mostly demonstrate PEA or asystole as the primary rhythm during cardiac arrest (DeBehnke*, et al.*, 1995; Kamohara*, et al.*, 2001; Tsai*, et al.*, 2012), it can be converted to ventricular fibrillation or tachycardia during chest compressions or after defibrillation (Vaagenes*, et al.*, 1997).

Evidence of myocardial and neurological injuries have been demonstrated in both VF and asphyxia animal models of cardiac arrest, although asphyxial models appear to create less altitude of myocardial dysfunction compared to the electrical shock model (Kamohara*, et al.*, 2001). It is believed that bradyasystole from asphyxiation requires less intense cardiac workloads and oxygen consumption compared to VF, and hence results in less myocardial damage. However, this observation was not confirmed by recent studies in rats (Tsai*, et al.*, 2012) and pigs (Wu*, et al.*, 2013). Using the rat model, Tsai and colleagues (2012) reported significant damage to myocardial tissues in both asphyxia and VF-induced models. A more diffuse injury was observed in the asphyxial model along with more severe mitochondrial damage. This severity, they concluded, was related to the period of hypoxia or incomplete ischaemia preceding cardiac arrest (Tsai*, et al.*, 2012). Furthermore, ROSC achievement in the asphyxial group was significantly lower (75% lower) than that of VF-induced animals (Tsai*, et al.*, 2012).

Brain damage is another feature of cardiac arrest animal models (Hickey & Painter, 2006; Hicks*, et al.*, 2000; Katz*, et al.*, 1995; Varvarousi*, et al.*, 2011) and is generally more pronounced in asphyxia compared to VF-induced cardiac arrest with comparable no-flow duration (Vaagenes*, et al.*, 1988; Vaagenes*, et al.*, 1997). However, asphyxia

less than five minutes shows little neuronal death (Kofler*, et al.*, 2004; Radovsky*, et al.*, 1997). The greater number of micro-infarcts in the asphyxial hypoxia model is believed due to differences in regional cerebral ischaemia (termed “trickle” flow) before cardiac arrest and complete cessation of blood flow in this model (Hickey & Callaway, 2005).

1.4 Determinants of resuscitation outcomes following cardiac arrest

Resuscitation outcome after cardiac arrest is defined as achievement of spontaneous (unassisted) circulation following cardiopulmonary resuscitation (CPR). The determinants of successful resuscitation include initial rhythm, cardiac arrest duration, the quality of CPR and coronary perfusion pressures (CPP) during CPR.

1.4.1 Initial ECG rhythm during cardiac arrest

The outcome from cardiac arrest resuscitation is closely correlated with the initial rhythm found at the scene (Kajino, et al., 2008; Kudenchuk, et al., 2012; Petrie, et al., 2001). In general, two types of fatal arrhythmias are pulseless VT/VF and bradyasystole (PEA/asystole) (Ornato & Peberdy, 1996).

Ventricular fibrillation (VF) was once believed to be the most fatal arrhythmia as it was found in most sudden death cases (Brouwer, et al., 2006; Huikuri, et al., 2001). However, today PEA/asystole-induced cardiac death is the most common (Cobb, et al., 2002) and increased from around 30% in the 1970s and 1980s (Myerburg*, et al.*, 1980; Stueven*, et al.*, 1989) to 60-85% in the last decade (Chen*, et al.*, 2011; Rea*, et al.*, 2004). Unfortunately, PEA/asystole are non-shockable rhythms, and therefore are associated with significantly lower chance of attaining ROSC and higher mortality rates during hospitalisation compared to cardiac arrest patients with VT/VF (Cohn*, et al.*, 2004; Mader*, et al.*, 2012; Meaney*, et al.*, 2010). Recently, Meaney and co-workers (2010) reported 65% VT/VF patients achieved ROSC compared to 42% with PEA/asystole. Moreover, the survival to hospital discharge was also significantly higher in VT/VF rhythm compared to PEA/asystole (37% vs 11%). A recent meta-analysis involving 204 clinical studies revealed what the authors termed “dismally low” long-term survivals in PEA/asystole arrest victims (0.2 - 4.7%) compared to 14 - 25% patients found in VF or VT (Sasson*, et al.*, 2010). Based on poor prognosis, initial PEA or asystole rhythm is as an independent predictor of lower chance of survival (Axelsson*, et al.*, 2012; Herlitz*, et al.*, 2005; Sandroni*, et al.*, 2007; Valenzuela*, et al.*, 1997). As mentioned earlier, a higher incidence of PEA/asystole is found in those

cardiac arrest victims precipitated by asphyxial hypoxia compared with those of a cardiac origin (Engdahl*, et al.*, 2003b). Therefore, new ways to resuscitate the heart in asphyxial-induced cardiac arrest are urgently required to improve survival outcomes.

1.4.2 Cardiac arrest duration

There is much experimental and clinical evidence showing that resuscitation outcome is correlated to cardiac arrest duration (Angelos*, et al.*, 2008; Campbell*, et al.*, 2007; Herlitz*, et al.*, 2006; Sladjana*, et al.*, 2011). The duration of cardiac arrest is generally referred as the time interval between the cardiovascular collapse to initial treatment either by bystander or emergency medical services (EMS). This definition is problematic during unwitnessed cardiac arrest as the time onset can only be estimated. However, it is shown that for each minute of resuscitation delay time, survival decreases by 10% (Valenzuela, et al., 1997). Higher survival rates for in-hospital compared to out-of-hospital cardiac arrest is associated with shorter times to definitive medical care (Fredriksson*, et al.*, 2010). This waiting period is also linked to reduced drug effects in OHCA regardless of its success in animal models (Reynolds*, et al.*, 2007).

The significance of cardiac arrest duration is further highlighted in a time-sensitive resuscitation model by Weisfeld and Becker (2002). This model proposed a three-phase cardiac arrest model consisting of electrical phase (<4 minutes cardiac arrest), circulatory phase (4-10 minutes), and metabolic phase (>10 minutes). According to this model, early defibrillation is only beneficial in the electrical phase, whereas, during the circulatory phase, chest compression with delayed defibrillation would be more valuable (Weisfeldt & Becker, 2002). In the metabolic phase, however, neither defibrillation nor chest compression could favourably improve survival, since global ischaemia and additional injuries have dispersed (Garza*, et al.*, 2009). In addition, epinephrine delivery at the metabolic phase mostly results in a more extensive myocardial dysfunction and brain damage (Angelos*, et al.*, 2008; Tang*, et al.*, 1995), which is a major problem in the pre-hospital clinical setting as most victims have been in cardiac arrest longer than 10 minutes before EMS arrival (Rittenberger*, et al.*, 2006). The importance of cardiac arrest duration on survival has also been emphasized by increased chance of survival in witnessed- compared to unwitnessed-arrest (Hostler*, et al.*, 2010).

1.4.3 Coronary perfusion pressure (CPP) during resuscitation

During cardiac arrest, profound hypoxia and ischaemia elicits systemic vasodilation including of the coronary vessels (Kern, 2000). It is believed that the dilation of coronary arteries at this state is maximal as the coronary resistance is negligible, and blood flow to the heart is driven by the pressure gradient between the aortic pressure and pressure in the right atrium (Kern, 2000). Thus, coronary perfusion pressure (CPP) during arrest is defined as the pressure gradient between aortic diastolic pressure and right atrial pressure (Kern, 2000; Paradis, et al., 1990). As the right arterial pressure is not significantly changed before and during arrest, CPP is mostly determined by aortic pressure during diastole when the heart fills with blood (Frenneaux & Steen, 2007).

In clinical cardiac arrest studies, CPP does not only correlate with myocardial perfusion, but also to the success of ROSC (Paradis*, et al.*, 1990). In 1990, Paradis and colleagues found that CPP above 15 mmHg during CPR appears to be a good predictor of spontaneous circulation recovery (Paradis, 1996; Paradis*, et al.*, 1990), whereas CPP below 15 mmHg is considered inadequate to facilitate myocardial flow (Frenneaux & Steen, 2007). Data from animal and human studies in overall support this contention (Berg*, et al.*, 2001; Martin*, et al.*, 1986; Yannopoulos*, et al.*, 2005), and when the arrest period is prolonged, much higher CPP threshold (>35 mmHg) is required to obtain ROSC (Reynolds, 2010.)

Paradis and colleagues further concluded that conventional CPR techniques were insufficient to increase CPP to sufficiently high levels to improve cardiac function (Paradis*, et al.*, 1990). They showed that only ~25% cardiac arrest patients had an initial CPP around 15 mmHg during CPR, while the remaining had CPP below 10 mmHg and failed to achieve ROSC. Alternatively, new CPR techniques using continuous chest compression *without rescue breathing* was found to improve CPP (Cunningham*, et al.*, 2012). This new method of resuscitation is known as cardio-cerebral resuscitation (CCR) and advocates no or minimal interruption in chest compression which appears to be important to maintain CPP (Ewy & Kern, 2009). The initiation of chest compressions, at least in the first five cycles, functions to increase the aortic-to-right atrial pressure gradient during cardiopulmonary resuscitation and build up stable CPP (Kern*, et al.*, 1988), and hence, each interruption costs a substantial period to reconstruct the optimal pressure (Kern, 2000). Thus, less interruption of chest compressions is shown to improve CPP during resuscitation (Berg*, et al.*, 2001), and increase successful ROSC compared to conventional CPR in

VF-induced arrest (Christenson*, et al.*, 2009; Vaillancourt*, et al.*, 2011). However, the efficacy of this technique is still debatable for asphyxial-induced cardiac arrest victims as profound hypoxia is the actual trigger of this type of cardiac arrest (Botran*, et al.*, 2011; Rea*, et al.*, 2010).

1.4.4 The quality of chest compressions

The effectiveness of chest compression is determined by its capability to force blood flow to the vital organs including the heart and the brain. The phases of compression-decompression separately drive blood through either coronary or systemic vasculatures (Kern, 2000). The coronary arteries are perfused during the decompression stage and the rest of the body through the compression phase (Berg*, et al.*, 2001). The current CPR guidelines have pointed out the importance of the depth (>5 cm) and the rate (>100 compressions/min) of chest compressions in an attempt to force adequate cardiac output to the body, followed by full recoil during the relaxation phase to ensure sufficient coronary perfusion (Nolan*, et al.*, 2010; Travers*, et al.*, 2010). This recommendation is based on clinical studies linking deeper chest compressions with better resuscitation outcomes (Babbs*, et al.*, 2008; Edelson*, et al.*, 2006). However, Hellevuo and colleagues caution against a compression depth >6 cm as this is associated with increased risk of sternal and rib fracture complications (Hellevuo*, et al.*, 2013).

Minimally interrupted CPR such in cardio-cerebral resuscitation has also been underlined in the most relevant CPR guidelines (Nolan*, et al.*, 2010; Travers*, et al.*, 2010). Clinical data has shown the method could enhance survival three times compared to conventional CPR (Bobrow*, et al.*, 2008; Ewy & Kern, 2009). In the case of asphyxial cardiac arrest, however, particularly for paediatric patients, artificial respiration is equally important as chest compression (Kleinman*, et al.*, 2010).

Although the importance of high-quality CPR has been emphasized in most guidelines, the overall translation into practice has been slow (Ødegaard*, et al.*, 2009). For example, the depth of chest compressions performed on OHCA victims was apparently suboptimal in >50% of cases using the 2005 guidelines, or >90% of cases based on 2010 guidelines (Stiell*, et al.*, 2012). In addition, slow compression rates (Abella*, et al.*, 2005a; Abella*, et al.*, 2005b) and incomplete recoil during resuscitation are still common (Fried*, et al.*, 2011). Moreover, prolonged CPR interruption is frequently seen in out-of-hospital, during transport and in-hospital settings (Abella*, et al.*, 2005a;

Olasveengen, *et al.*, 2008; Wik, *et al.*, 2005). All these deviations from the guidelines could contribute to the current suboptimal outcomes (Kern, *et al.*, 1988; Paradis, *et al.*, 1990).

1.5 Post-cardiac arrest syndrome

According to Nolan and colleagues (2008), the four major components of post-cardiac arrest syndrome are neurological injury, myocardial dysfunction, systemic ischaemia/reperfusion response (i.e. systemic inflammation, profound vasodilation and coagulopathy), and persistent precipitating pathology. The following sections will discuss post-arrest myocardial dysfunction, neurological damage and coagulopathy.

1.5.1 Post-arrest myocardial dysfunction

It has been estimated that over 50% of cardiac arrest patients die from myocardial failure despite restored circulation (El-Menyar, 2005). Myocardial dysfunction develops during the early phase of resuscitation, which mostly leads to persistent haemodynamic instability within hours of hospital admission (Chalkias & Xanthos, 2012; Kern, *et al.*, 1997; Kern, *et al.*, 1996).

Myocardial dysfunction appears to be a reversible stunning effect of the heart (not irreversible cell death as in myocardial infarction) and this manifests in reduced systolic and diastolic function within minutes of ROSC and coronary reperfusion (Mongardon, *et al.*, 2011). The stunning effect is associated with impaired contractility, decreased cardiac output, reduced ventricular work capacity, reduced cardiac compliance, and reduced end-diastolic volume (Gazmuri, *et al.*, 2008; Gazmuri, *et al.*, 1996; Kern, *et al.*, 1996; Tang, *et al.*, 1993). Stunning can resolve within four hours or it can persist longer for days following resuscitation (Gazmuri, *et al.*, 1996; Kern, *et al.*, 1996; Laurent, *et al.*, 2002). If low cardiac output continues for more than 24 hours, it could lead to compromised tissue oxygen delivery, multiple organ failure and death (Laurent, *et al.*, 2002).

The mechanism underlying post-arrest myocardial stunning is controversial. Two major factors include excessive catecholamine release and an uncontrolled production of oxygen free radicals during reperfusion (Chalkias & Xanthos, 2012). Catecholamine surge emerges in early ROSC even without vasopressor administration during resuscitation (Lathers, *et al.*, 1989; Niemann & Garner, 2005; Prengel, *et al.*, 1992;

Schoffstall, *et al.*, 1990). Regardless, systemic hypotension is still apparent during this hyper-adrenergic state (Chalkias & Xanthos, 2012). The high catecholamine concentration is notably correlated to myocardial depression (Angelos, *et al.*, 2008; Niemann & Garner, 2005; Tang, *et al.*, 1995), which may result from catecholamine-provoked calcium overloading and loss of sensitivity (Wittstein, *et al.*, 2005). The high production of oxygen free radicals is also associated with reduced ATP generation, impaired electrolyte exchange and dysregulation of adrenergic signalling itself (Ayoub, *et al.*, 2003; Goldhaber & Qayyum, 2000). Fluid shift and cellular swelling may also occur and lead to myocardial wall thickening with myocardial depression early following ROSC (Chalkias & Xanthos, 2012; Lazar, *et al.*, 1985). Oxygen free radicals further result in mitochondrial and endothelial damage (Fink, *et al.*, 2010). For example, re-oxygenation markedly reduces endothelial nitric oxide synthase (eNOS) level (Gulyaeva, *et al.*, 1996), and thereby, nitric oxide (NO) production; meanwhile, the level of the vasocontrictive mediator, endothelin, increases (Entman & Smith, 1994; Hansen, 1995). Consequently, there is a loss of endothelial-dependent vasoregulation (Adams, 2006), which further exacerbates post-resuscitation myocardial dysfunction (Ferrari & Hearse, 1997).

1.5.2 Post-arrest neurological dysfunction

Neurological dysfunction affects more than 80% of cardiac arrest victims (Madl & Holzer, 2004) and contributes to two thirds of out-of-hospital and one third of in-hospital arrest mortality (Laver, *et al.*, 2004). Neurological damage occurs from hypoxia and ischaemia during the arrest, and reperfusion injury during re-oxygenation after ROSC (Lundbye, *et al.*, 2012). Unlike myocardial dysfunction, brain damage predominantly causes delayed death at later times up to seven days after ROSC (Horn & Schlote, 1992; Petito, *et al.*, 1987).

The duration and severity of brain ischaemia affects specific different parts of the neuronal population. Although the appearance of DNA degradation as a marker of cellular death is evident after ten hours to three days following brain ischaemia (Beilharz, *et al.*, 1995), in experimental studies Kawai, *et al.* (1992) has shown that early histopathological changes occur as soon as 15 min following five minute cardiac arrest-induced global ischaemia. These early neuronal changes mainly affect the cortical layers, nucleus caudatus, hippocampus, and nucleus reticular thalamic (NRT) area, showing the presence of scattered dark neurons surrounded with cytoplasmic halos (Kawai, *et al.*, 1992). Peripheral compartments (vacuoles) start to appear mainly

in neurons of the NRT and CA1 hippocampal area after one hour post-insult, while the cerebral cortex, striatum and pars reticulate of substantia nigra show the presence of pyknotic neurons. After six hours, neuronal cell loss may develop in the cortical and NRT area, while shrunken, dark and pyknotic neurons are increasingly conspicuous in CA1 hippocampus. When observation continues to 24 hours, neuronal loss becomes more apparent in NRT, cortex and CA1 hippocampus(Kawai*, et al.*, 1992).

Neuronal death occurs from both apoptosis and necrosis (Ünal-Çevik*, et al.*, 2004). Apoptosis or "programmed cell death" is characterized by cellular shrinkage and chromatin condensation, whereas necrosis appears with cellular swelling and membrane lysis (Harukuni & Bhardwaj, 2006). While necrosis predominantly occurs in severe and prolonged ischaemia, apoptosis endures during brief ischaemia. (Ünal-Çevik*, et al.*, 2004). This is evident using DNA end-labelling showing abundant apoptotic morphology after 15 min brain ischaemia and profound necrotic death after 60 min (Beilharz*, et al.*, 1995).

1.5.3 Post-arrest coagulopathy

The severity of myocardial stunning and neurological damage after cardiac arrest is also greatly influenced by systemic inflammation, hypotension and impaired coagulation (Adrie*, et al.*, 2004; Jones*, et al.*, 2008). These responses are believed to be predominantly evoked by hypoxic and ischaemic-related endothelial injury (Boyle Jr*, et al.*, 1996), which negatively affects whole body homeostasis (Adams, 2006).

As early as the 1970s, intravascular coagulation was found in the capillaries of the pulmonary and renal microcirculation in patients suffering from cardiac arrest who underwent CPR (Hartveit & Halleraker, 1970), and microthrombi were shown to occur in cerebral vessels five to ten minutes after cardiac arrest (Bottiger*, et al.*, 1995). Animal studies confirmed these clinical findings and hypothesised that a marked activation of blood coagulation after cardiac arrest and CPR may be part of the reperfusion phenomenon responsible for mortality (Bottiger*, et al.*, 1995; Johansson*, et al.*, 2003). Bottinger and colleagues further showed a marked activation of blood coagulation and fibrin formation after prolonged cardiac arrest and CPR in humans and presented evidence that this was not balanced adequately by the activation of fibrinolysis (Bottiger*, et al.*, 1995; Böttiger*, et al.*, 2002). Gando and colleagues also confirmed a coagulopathy post cardiac arrest in humans and reported an increase in fibrin formation without sufficient anti-coagulation pathways (Gando*, et al.*, 1997;

Gando*, et al.*, 1999). This coagulopathy state was also confirmed in experimental cardiac arrest (Bottiger*, et al.*, 1995; Johansson*, et al.*, 2003). The spiralling pro-coagulation state is more commonly detected in OHCA patients three hours post-resuscitation, simultaneously with augmented inflammatory markers (Adrie*, et al.*, 2005).

As more studies were carried out using new methodologies to assess coagulopathy in plasma and whole blood the complexity of coagulopathy post-cardiac arrest was apparent. For example, Adrie*, et al.* (2005) showed a paradoxical prolongation in prothrombin time (PT) and activated partial thromboplastin time (aPTT), not a shortening as would be expected if the blood was clotting more rapidly than normal. PT and aPTT are indicators of the contribution of the extrinsic and intrinsic pathways to blood clotting respectively (see Fig. 1.3), and prolonged PT and aPTT indicate a hypocoagulation state. Interestingly, based on biomarker measurement, Adrie's study also revealed a considerable increase in coagulation activity along with depressed anticoagulation, highlighting a hypercoagulation state (Adrie*, et al.*, 2005). Similar discrepancies were found between the suppressed fibrinolysis markers (Bottiger*, et al.*, 1995; Gando*, et al.*, 1997; Geppert*, et al.*, 2001) and the appearance of hyperfibrinolysis in two independent clinical studies (Schöchl*, et al.*, 2013; Viersen*, et al.*, 2012). Schochl's group (2013) and Viersen's laboratory (2012) reported the appearance of hyperfibrinolysis in 30%-50% cardiac arrest cases upon admission to hospital using a different whole blood coagulation assessment method known as rotational thromboelastometry (ROTEM). The disparities seen in these studies are possibly predisposed by the perfusion status when the blood samples were withdrawn.

The ongoing coagulopathy disparities were assessed in a pig model by White and colleagues (2011) who proposed the presence of different and distinctive coagulation states at different times from the onset of arrest, resuscitation (CPR) through to post-ROSC (reperfusion). During no-flow (arrest), coagulation status was characterized by shorter clotting times with increased clot amplitudes, suggesting increased coagulation. In contrast, during hypoperfusion (CPR and early reperfusion), hypocoagulation appears to be more pronounced (White*, et al.*, 2011). While the subject of post-arrest coagulopathy is rapidly evolving as more studies are being performed, it seems that the whole body physiological state of hypoperfusion, instead of the severity of injury, is an independent predictor of coagulopathy in trauma and cardiac arrest patients (Brohi*, et al.*, 2007a; Brohi*, et al.*, 2008; Hess*, et al.*, 2008; Lustenberger*, et al.*, 2010). There is a major unmet need to correct coagulopathy and the pro-inflammatory state post-

cardiac arrest, which may assist to reduce or prevent myocardial dysfunction and brain injury (Kim, *et al.*, 2013).

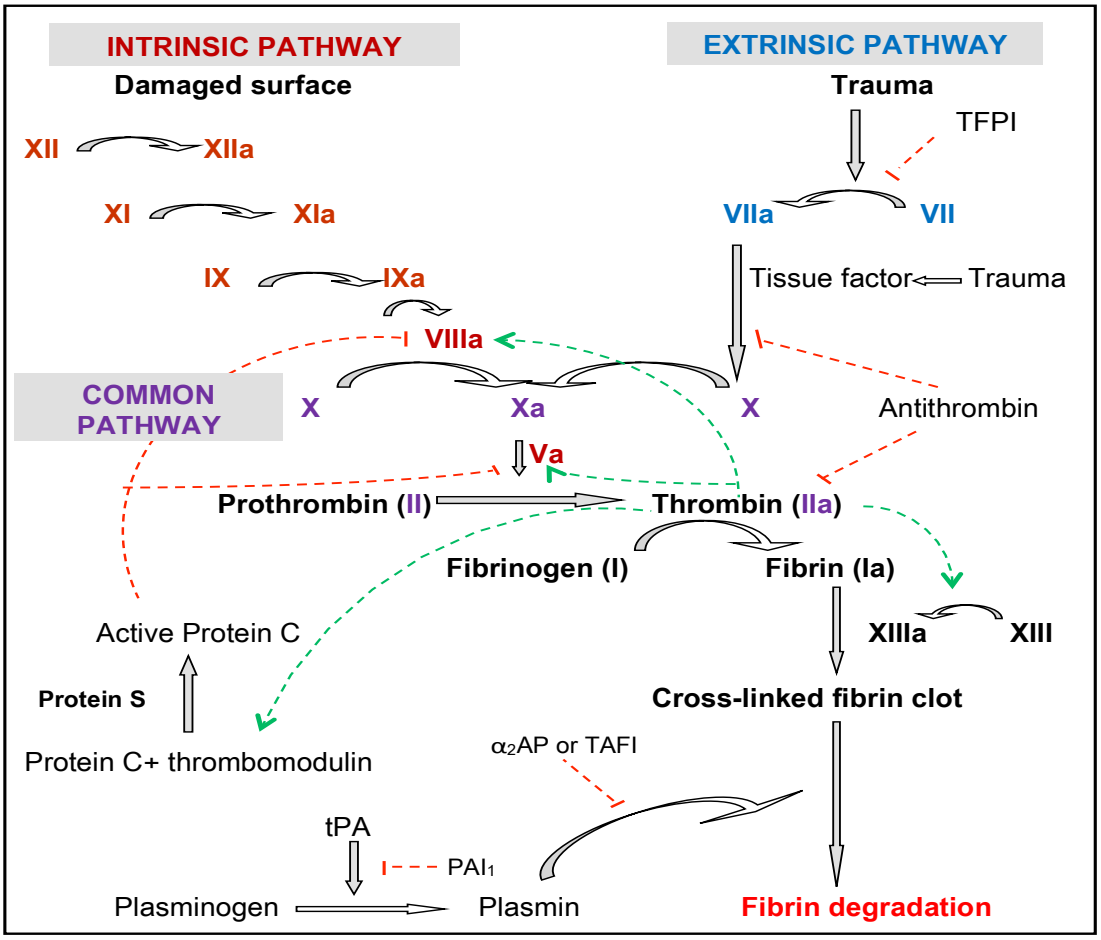


Figure 1.2 Extrinsic and intrinsic coagulation pathways

Both extrinsic and intrinsic pathways serve to activate common pathway factor X to factor Xa, which in turn converts prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin and activates factor XIII to XIIIa to stabilise the fibrin clot. Clot formation can be inhibited by tissue factor pathway inhibitor *(*TFPI) through factor VII inactivation, or by antithrombin through thrombin or factor X inactivation. Activated protein C also acts as an anticoagulant by deactivation of factor VIIa and Va. The clotting pathway is balanced by the fibrinolytic system, which is initiated with tissue plasminogen activator (tPA)-induced plasminogen conversion to plasmin, and in turn leads to fibrin degradation. Fibrinolysis can be inhibited by plasma activator inhibitor (PAI1), alpha2-antiplasmin (α_2AP) or thrombin activatable fibrinolysis inhibitor (TAFI). Prothrombin time (PT) assesses coagulation factors in **blue**, activated partial thromboplastin time (aPTT) assesses factors in **red**, while both measure factors in **purple** (common pathway). The figure is adapted from Cito, et al. (2013).

1.6 Standard management of cardiac arrest: the role of epinephrine

Cardio-pulmonary resuscitation (CPR) is performed to facilitate blood circulation in cardiac arrest victims, whereas, defibrillation is applied to convert potentially fatal cardiac "shockable" arrhythmias to sinus rhythm. Together with CPR, early defibrillation has been shown to promote survival up to three-fold (Stiell*, et al.*, 2004; Weisfeldt*, et al.*, 2010). However, over the past 50 years survival rates have only increased from 0% to 1-10% (Kramer-Johansen, 2007) and the efficacy of defibrillation appears only useful in VF-induced cardiac arrest (Steen*, et al.*, 2003) and not for PEA/asystole (Hallstrom*, et al.*, 2007). Current resuscitation guidelines recommend the use of epinephrine after two consecutive unsuccessful defibrillation attempts for VF/VT-induced cardiac arrest or immediately during CPR (every 3-5 min) for PEA/asystole (see Fig. 1.4) (Kleinman*, et al.*, 2010; Neumar*, et al.*, 2010).

Epinephrine was first introduced to resuscitation science in the 1960s, and there has been a general consensus that epinephrine improves resuscitation outcomes (Gueugniaud*, et al.*, 2000). During CPR, chest compressions only increase coronary perfusion to <25% of normal perfusion (Voorhees*, et al.*, 1980; Weil*, et al.*, 1985) and epinephrine adds to this by improving and maintaining coronary and cerebral perfusion by increasing aortic diastolic pressure and coronary perfusion pressure that is important to produce ROSC (Krismer*, et al.*, 2000; Paradis*, et al.*, 1991). Epinephrine elicits peripheral vasoconstriction through α-adrenergic receptor, which some argue helps force blood to the vital organs (Paradis*, et al.*, 1990; Reynolds*, et al.*, 2010). These potential benefits have received much experimental support from animal studies (Cairns & Niemann, 1998; Chen*, et al.*, 2010; Chen*, et al.*, 2006; Kitsou*, et al.*, 2009; Lindner*, et al.*, 1991; Michael*, et al.*, 1984; Stroumpoulis*, et al.*, 2008; Zuercher*, et al.*, 2011). Accordingly, epinephrine administration has become the gold standard drug in resuscitation until the present day, despite a lack of definitive evidence of benefits in clinical trials.

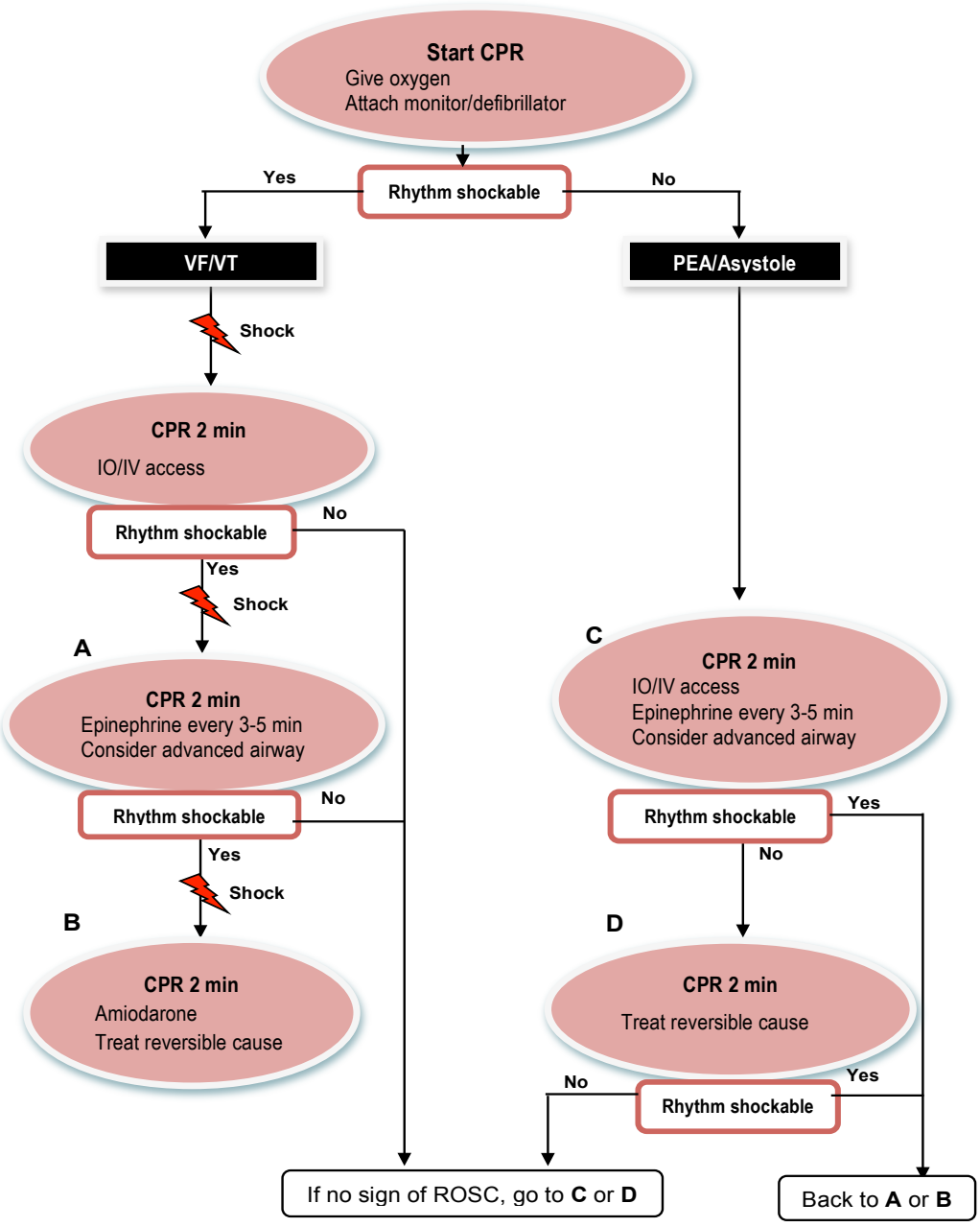


Figure 1.3 Diagram of Advanced Cardiac Life Support (ACLS) algorithm
Based on AHA guidelines for cardiac arrest 2010 (Neumar, *et al.*, 2010)

However, epinephrine also has adverse effects (Behringer*, et al.*, 1998; Herlitz*, et al.*, 1995; Mitchell*, et al.*, 2000; Stiell*, et al.*, 1992; Woodhouse*, et al.*, 1995). It increases myocardial oxygen consumption (Ditchey & Lindenfeld, 1988), increases post-resuscitation myocardial dysfunction (Tang*, et al.*, 1995) and is pro-arrhythmic (Tovar & Jones, 1997). High and repetitive doses of epinephrine have also been reported to decrease microcirculation to the superficial areas of the brain (Ristagno*, et al.*, 2009a), induce ischaemia in underperfused organs (e.g. gastrointestinal tract) (Studer*, et al.*, 2002), stimulate coagulopathy by causing platelet aggregation (Hjemdahl*, et al.*, 1994), and exacerbate the pro-inflammatory response (Ito*, et al.*, 2001).

Thus, while it has been recognised epinephrine may be beneficial to produce ROSC, it does not necessarily assure long-term survival and may in fact be harmful in some cardiac arrest patients. If epinephrine fails during CPR in both adults and children, other vasopressors and antiarrhythmics are recommended in the CPR guidelines, including vasopressin, amiodarone, lidocaine and magnesium (Neumar*, et al.*, 2010). Unfortunately, like epinephrine, they all failed to increase survival outcomes in clinical trials (Charalampopoulos & Nikolaou, 2011; Ewy & Sanders, 2013; Olasveengen*, et al.*, 2009a). In summary, the current value of epinephrine in reducing the post-resuscitation syndrome appears to depend on the dosage given, the timing of administration, and the initiating factor of the cardiac arrest.

1.7 Standard management of cardiac arrest: mild therapeutic hypothermia

In the past few years, therapeutic hypothermia (32-33^{o}C) has been introduced to target a hypometabolic state in cardiac arrest patients (Bergman*, et al.*, 2010; Safar*, et al.*, 1996). In fact, this strategy is now considered as the only therapy that is clinically proven to improve neurological function following cardiac arrest (Ferreira*, et al.*, 2009; Nolan*, et al.*, 2008).

1.7.1 Therapeutic hypothermia: neurological and cardiovascular protection

Hypothermia is clinically defined as a core temperature <35^{o}C (Luscombe & Andrzejowski, 2006). The different grades of hypothermia can be classified as mild hypothermia (32-34^{o}C), moderate hypothermia (28-32^{o}C), deep hypothermia (11-28^{o}C), profound hypothermia (6-11^{o}C), and ultra-profound hypothermia (<6^{o}C) (Varon & Acosta, 2008). It may occur accidentally as body temperature regulation is overwhelmed by certain stressors, or intentionally induced for therapeutic purposes

(Prisco*, et al.*, 2012). The concept of therapeutic hypothermia was introduced in cardiac surgery in the 1950s to protect the heart and vital organs from ischaemic insult (Bigelow, 1959). It has been regularly applied in surgical procedures that require prolonged ischaemic periods, such as in open heart and transplantation surgery and neurosurgery (Lampe & Becker, 2011). Currently, therapeutic hypothermia is gaining popularity in emergency units for treating ongoing ischaemia, such as for traumatic brain injury, stroke, haemorrhagic shock or cardiac arrest (Marion & Bullock, 2009).

Improved neurological function with hypothermia is believed to occur through several mechanisms. Hypothermia reduces brain metabolism, leading to better ATP preservation and limiting toxic by-products (Lanier, 1995), including lactate (Yager & Asselin, 1996), ion H^+, and ROS (Globus*, et al.*, 1995). For every 1^oC drop in body temperature there is a 6-10% drop in cerebral metabolic rate (Ehrlich*, et al.*, 2002; Hägerdal*, et al.*, 1975; Milde, 1992; Small*, et al.*, 1999). Based on that, deep and even profound hypothermia is preferred to provide protection during prolonged circulatory arrest (Capone*, et al.*, 1996; Tisherman*, et al.*, 1991; Tisherman*, et al.*, 1990). Neuroprotection is also linked to reduced intracellular Ca^{2+} overloading (Mitani*, et al.*, 1991; Takata*, et al.*, 1997), reduced glutamate and dopamine release (Globus*, et al.*, 1995; Hachimi-Idrissi*, et al.*, 2004) and reduced NO-induced excitotoxicity (Kader*, et al.*, 1994). In addition, hypothermia could improve cell membrane permeability, preventing brain oedema and intracellular acidosis (Polderman, 2009).

Mild hypothermia also improves cardioprotection (Schwartz*, et al.*, 2012). Cardiac output decreases by 6-7% with every 1^oC temperature loss (Bernard*, et al.*, 1997), indicating a better energy/oxygen preservation in colder temperatures. Mild (32-35^oC) hypothermia has been consistently shown to reduce infarct size and improve cardiac function in experimental ischaemic myocardium (Hale & Kloner, 2011; Tissier*, et al.*, 2012).

1.7.2 Therapeutic hypothermia in cardiac arrest settings

Therapeutic hypothermia has been a recommended strategy for post-cardiac arrest management since 2003 (Nolan*, et al.*, 2003), and appeared in the AHA resuscitation guidelines in 2005 (Nolan*, et al.*, 2005). This recommendation is based on two randomised clinical trials showing neurological benefits of hypothermia induced following cardiac arrest compared with the normothermic group (Bernard*, et al.*, 2002; The Hypothermia After Cardiac Arrest Study Group, 2002). Mild therapeutic

hypothermia was associated with improved short-term recovery, shorter intensive care unit (ICU) stays, and improved long term outcomes (Cronberg*, et al.*, 2009; Holzer*, et al.*, 2005; Nielsen*, et al.*, 2009; Storm*, et al.*, 2008).

Despite the clinical benefits, there remain some concerns with lower temperatures. Hypothermia can lead to depressed cardiac function and arrhythmias, especially when the body temperature drops below 30^{o}C (Leonov*, et al.*, 1990; Piktel*, et al.*, 2012; Schwartz*, et al.*, 2012; Weinrauch*, et al.*, 1992; Wong, 1983). Pulmonary oedema, depressed immune function, increased plasma amylase, electrolyte imbalance, insulin resistance and hypovolaemia are among complications frequently found with hypothermia therapy (Polderman, 2004; Schubert, 1995). One of the most concerning adverse effects of hypothermia below 33^{o}C is coagulopathy (Polderman, 2004) with increased bleeding time (Valeri*, et al.*, 1995). Mild hypothermia (34-35^{o}C), however, is considered relatively safe, unless the patients are at a high risk of bleeding (Batista*, et al.*, 2010; Lampe & Becker, 2011; Oddo*, et al.*, 2006).

1.7.3 Therapeutic hypothermia on location: intra-arrest application

A new application of therapeutic hypothermia to improve cardiac arrest outcomes is the administration of hypothermia as close to the point of injury as possible (Garrett*, et al.*, 2011). Although the mode, timing and rate of cooling and rewarming remain controversial, hypothermia *prior to ROSC* has been shown to be feasible in animal and human studies (Scolletta*, et al.*, 2012). In addition, the emerging data suggest improvements in ROSC achievements, and neurological and cardiovascular functions with intra-arrest hypothermia compared with hypothermia and/or normothermia post-ROSC (Menegazzi*, et al.*, 2009; Nozari*, et al.*, 2006; Tsai*, et al.*, 2008; Zhao*, et al.*, 2008).

Hypothermia induced early *during* the ischaemic period rather than *after* reperfusion in an ischaemic-reperfusion model has been shown to reduce infarct size (Dae*, et al.*, 2002; Gotberg*, et al.*, 2008; Hale & Kloner, 1997; Hamamoto*, et al.*, 2009; Otake*, et al.*, 2007; Yannopoulos*, et al.*, 2009). In a swine model of VF-induced cardiac arrest, early hypothermia significantly improved CPP during resuscitation, reduced epinephrine dose required, enhanced rate of defibrillation success, and improved 96 hr survival compared to normothermic arrest (Boddicker*, et al.*, 2005; Tsai*, et al.*, 2008). Interestingly, these benefits were not significant, however, if hypothermia was applied following ROSC (Tsai*, et al.*, 2008). Improved ROSC success is associated with slower

ECG decay during VF with intra-arrest hypothermia (Menegazzi*, et al.*, 2009). In asphyxial-induced cardiac arrest models, intra-arrest hypothermia is shown to increase ROSC achievement compared to the normothermic group (78% vs 45%), although, the benefit on survival is considered insignificant (Albaghdadi*, et al.*, 2010). Importantly, intra-arrest hypothermia resulted in better neurological outcomes compared to post-arrest and normothermic groups (Xiao*, et al.*, 1998).

1.8 Alternative therapeutic strategies to improve resuscitation outcome are urgently needed

As already discussed, a major drawback with current vasopressor use is the increased severity of post cardiac arrest syndrome (Hagihara*, et al.*, 2012; Olasveengen*, et al.*, 2012), partly due to increased myocardial oxygen consumption. Alongside mild hypothermia, therapeutic strategies to improve post-resuscitation outcomes are urgently required. Such strategies may include haemodynamic-directed therapy (Jones*, et al.*, 2008), coronary intervention (Batista*, et al.*, 2010), extracorporeal life support (Kagawa*, et al.*, 2010), and alternative methods of delivering CPR (Ewy & Sanders, 2013).

Another new drug strategy that may improve resuscitation outcomes is intravenous (or intraosseous) administration of adenosine and lidocaine (AL), which is the topic of the present thesis. The idea is based on the clinical use of the drug combination to resuscitate the heart after cardiac surgery. At high concentrations, AL is a cardioplegia that arrests and protects the heart to allow the surgeon to perform the operation (Dobson*, et al.*, 2013; Dobson & Jones, 2004). At lower therapeutic doses, AL has been shown to hypotensively resuscitate rats and pigs after 60-90 min of shock and up to 75% of blood loss with improved cardiac, haemodynamic and whole body function (Granfeldt*, et al.*, 2012; Letson & Dobson, 2011b). In addition, in the presence of magnesium, AL corrects coagulopathy after haemorrhagic shock (Letson*, et al.*, 2012). What follows is a brief introduction into the properties of each drug followed by the work carried out on AL in cardiac surgery and different trauma states, and finally the hypothesis to be tested in the asphyxial hypoxia model of cardiac arrest in the rat.

1.8.1 Adenosine as a potential candidate for cardiac arrest treatment

Adenosine is a ubiquitous purine signalling nucleoside that is continuously synthesised and metabolised intracellularly and extracellularly to maintain its normal tissue concentration of 30-300 nM (Cohen & Downey, 2008; Eltzschig, 2013). The level of tissue adenosine and its extracellular release increases during cellular distress, including hypoxia or ischaemia; and this is believed to be due to increased adenosine synthesis, inhibition of adenosine reuptake from the cell surface and increased activities of extracellular ecto-nucleotidase, the enzyme responsible for adenosine breakdown (Cohen & Downey, 2008; Schulte & Fredholm, 2003).

The main physiological function of adenosine is to help match oxygen demand and supply during hypoxia and ischaemia, particularly in the heart (Berne, 1980). In 1970, Olsson demonstrated that a 15 second left coronary artery occlusion in dogs triggered a six-fold increase in myocardial adenosine (Olsson, 1970), which was consistent with Katori and Berne's earlier hypothesis of adenosine increasing coronary flow (Katori & Berne, 1966). Adenosine may also reduce vascular tone by modulating sympathetic neurotransmission (Tabrizchi & Bedi, 2001). In addition to increasing blood flow, adenosine also lowers energy demand by activating a number of intracellular survival kinase pathways (Cohen & Downey, 2008; Hausenloy & Yellon, 2006; Headrick & Peart, 2005).

Apart from matching supply to demand, adenosine is an anti-arrhythmic (Canyon & Dobson, 2004; Wilbur & Marchlinski, 1997) and is known to have potent anti-inflammatory, immunomodulatory and anti-coagulation functions (Ramakers*, et al.*, 2011; Rocha Lapa*, et al.*, 2012; Sitkovsky*, et al.*, 2004). It acts as a broad spectrum, cytoprotective, multi-modulator in response to stress to an organ or tissue (Fredholm, 2007; Grenz*, et al.*, 2011), which makes it a possible candidate for cardiac arrest studies. One possible drawback, however, is that adenosine has a short half-life in whole blood of less than 10 seconds (Lerman & Belardinelli, 1991) as it is rapidly cleared from the circulation via cellular uptake, primarily by erythrocytes and vascular endothelial cells (Belardinelli, 1993; Gerlach*, et al.*, 1987).

1.8.1.1 The role of adenosine receptors

The cardiovascular effects of adenosine, are mediated through multiple G protein-coupled adenosine receptors (A_1, A_{2A}, A_{2B} and A_3 receptors) and these receptors are

coupled to a wide range of secondary messenger cascades (Chen*, et al.*, 2013; Vinten-Johansen*, et al.*, 1999; Wilson*, et al.*, 2009). Adenosine receptors are classified based on their ability to inhibit (A_1 or A_3) or stimulate ($A_{2A,B}$) the activity of adenylyl cyclase (Shryock & Belardinelli, 1997). In the cardiovascular system, the A_1 and A_3 receptors are located predominantly on cardiomyocytes, while A_{2A} and A_{2B} receptors are generally found on the endothelium and vascular smooth muscle of the coronary arteries (Cohen & Downey, 2008; Fredholm*, et al.*, 2000; Headrick & Peart, 2005). A_1 receptors are also found in some arterioles, particularly in the kidney (Fredholm*, et al.*, 2000).

It is widely accepted that A_1 receptor activation in myocytes leads to demand-lowering or negative chronotropic, dromotropic and inotropic as well as antiadrenergic effects (Cohen & Downey, 2008; Vinten-Johansen*, et al.*, 1999). A_1 receptors are also known to open K_{ATP} channels and stimulate preconditioning in cardiomyocytes (Liang, 1996). The activation of A_{2A} and A_{2B} receptors results in vasodilatation in a range of vasculature, including aorta and coronary beds (Fahim*, et al.*, 2001; Fredholm*, et al.*, 2000). In addition, A_{2A} receptor activation has anti-platelet aggregation and anti-inflammatory properties (Chen*, et al.*, 2013), while A_{2B} activation plays a role in adenosine-mediated inhibition of cardiac fibroblasts, stimulation of NO production and angiogenesis (Jacobson & Gao, 2006). Meanwhile, similar to A_1 receptors, A_3 receptor stimulation is also important during ischaemia (Jacobson & Gao, 2006). It elicits cardioprotection through activation of K_{ATP} channels and/or RhoA–phospholipase D (PLD) (Mozzicato*, et al.*, 2004; Zhao & Kukreja, 2002), and potentially exerts cytoprotective effects through MAPK or ERK1/2 coupling (Headrick & Peart, 2005; Liang & Jacobson, 1998). Accordingly, activation of specific adenosine receptors, either directly or indirectly, becomes promising therapeutic targets for a wide range of diseases, including cerebral and cardiovascular diseases (Chen*, et al.*, 2013; Jacobson & Gao, 2006).

1.8.1.3 Adenosine as an anti-arrhythmic agent

The anti-arrhythmic properties of adenosine are thought to be elicited by A_1 receptors located in the SA node, AV node and atrial myocytes (Wilbur & Marchlinski, 1997). Adenosine A_1 receptor activation results in potassium channel ($I_{K^+_{ADO,\,Ach}}$) opening, leading to hyperpolarization way below the resting membrane potential. As a result, a higher threshold is required to fire following the action potential, thereby preventing myocardial over-excitation (Wilbur & Marchlinski, 1997). Additionally, adenosine could

reduce the incidence of arrhythmias indirectly through its antiadrenergic action (Biaggioni*, et al.*, 1991). However, like many anti-arrhythmic agents, adenosine under some conditions can be pro-arrhythmic (Camaiti*, et al.*, 2001; Pelleg*, et al.*, 2002; Romer & Candinas, 1994). Despite this, adenosine is a widely used FDA-approved cardiac drug for the treatment of paroxysmal supraventricular tachycardia (PSVT) including for critically ill infants and children (Holdgate & Foo, 2006; Rossi*, et al.*, 1992).

1.8.1.2 Adenosine as a cardioprotectant

The cardioprotective role of adenosine has been indicated in a large number of experimental myocardial ischaemia and infarction studies (Cohen & Downey, 2008; Kin*, et al.*, 2005; Sivaraman & Yellon, 2014; Vinten-Johansen*, et al.*, 1999). Experimentally infusion of adenosine or an adenosine A_1 agonist has been shown to significantly reduce myocardial infarct size and post-reperfusion myocardial dysfunction in a number of animal models (Liu*, et al.*, 1991; Ogawa*, et al.*, 1996; Sekili*, et al.*, 1995; Toombs*, et al.*, 1992; Xu*, et al.*, 2000). Unfortunately, these animal studies have not translated to treat clinical myocardial ischaemia or infarction (Kloner*, et al.*, 2006; Ross*, et al.*, 2005). Reasons for lack of translation are related to the complexity of the human conditions and the multiple disease processes that accompany a myocardial event (Bolli*, et al.*, 2004; Miura & Miki, 2008).

Adenosine has also been shown to be involved in an innate protection mechanism of the heart known as ischaemic preconditioning (IPC) (Headrick, 1996; Vinten-Johansen*, et al.*, 2007). IPC was first described in 1986 by Murry, Jennings and Reimer who reported a ~75% reduction in infarct size in anaesthetised open-chested dogs after three brief episodes of ischaemia-reperfusion followed by 40 min left coronary occlusion (Murry*, et al.*, 1986). Two different time frames of preconditioning have been identified; an early window, which lasts one to three hours, and a “delayed” window, which develops over many hours and can last up to three days (Baxter, 2002; Bolli, 2000). Adenosine has been implicated in 'triggering' both early and delayed IPC (Baxter, 2002; Yang*, et al.*, 2010b). More recently, it was found that brief episodes of ischaemia-reperfusion following ischaemia within the first few minutes after reperfusion also reduces infarct size and adenosine is believed to be involved in this natural cardioprotective strategy (Vinten-Johansen & Shi, 2011). Thus, adenosine appears to be a very ancient mechanism of protection and conditioning of the heart during and

following short episodes of sublethal ischaemia-reperfusion (angina) (Vinten-Johansen*, et al.*, 2007).

1.8.1.4 Adenosine as an anti-inflammatory and anti-thrombolytic agent

Adenosine is a potent endogenous anti-inflammatory drug, with mechanisms including the inhibition of neutrophil activation, tumor necrosis factor-alpha expression, cytokine release, and platelet adhesion, (Bouma*, et al.*, 1997; Cronstein, 1994; Wagner*, et al.*, 1998). The broad anti-inflammatory effects of adenosine involve activation of adenosine receptors, primarily the A_{2A} adenosine receptors (Haskó & Pacher, 2008; Lappas*, et al.*, 2005), which are predominantly expressed in most inflammatory cells, including neutrophils, macrophages, T lymphocytes, dendritic cells, mast cells, endothelial cells and platelets (Haskó & Pacher, 2008). The activation of A_{2A} receptors has been shown to inhibit neutrophil responses, including the release of reactive oxygen species and elastase, and expression of adhesion molecules (Lappas*, et al.*, 2005; Sullivan, 2003). In platelets, adenosine A_{2A} activation inhibits the mobilization of intracellular Ca^{2+} and influx of external calcium, both associated with activation of adenylate cyclase, leading to the inhibition of platelet adhesion (Johnston-Cox & Ravid, 2011). Meanwhile, A_{2B} receptors have been implicated in vascular inflammation and macrophage deactivation (Haskó*, et al.*, 2009; Xaus*, et al.*, 1999; Yang*, et al.*, 2008; Yang*, et al.*, 2006) as well as inhibition of platelet aggregation (Yang*, et al.*, 2010a). More recently, adenosine's anti-inflammatory effect was shown to include A_3 receptors, which are highly expressed in inflammatory cells (Bar-Yehuda*, et al.*, 2007). An increasing number of studies have shown that A_3 activation with specific agonists induces a down-regulation of the NF-κB signalling pathway in inflammatory cells and the initiation of protective immune-modulatory effects (Bar-Yehuda*, et al.*, 2007; Fishman*, et al.*, 2012). Thus, it is possible that these multi-modulatory properties and adenosine receptor targets may be of some benefit in cardiac arrest patients as systemic inflammation and thrombosis are implicated in post-resuscitation syndrome.

1.8.2 Lidocaine as a potential candidate for cardiac arrest treatment

1.8.2.1 Lidocaine as a local anaesthetic and an antiarrhythmic agent

Lidocaine was first used clinically as a local anaesthetic in the 1940s since it effectively inhibits cellular depolarisation through sodium (Na^+) channel inactivation (Holmes, 1969). Starting in 1950, lidocaine was increasingly used to prevent left ventricular

arrhythmias (Gianelly*, et al.*, 1967; Lie*, et al.*, 1974; Southworth*, et al.*, 1950) in emergency departments, and during and after cardiac surgery (Rosen*, et al.*, 1975). Lidocaine's antiarrhythmic action is mediated by its inhibition on voltage-dependent Na^+ fast channels in the inactivated state (Vedantham & Cannon, 1999). At more depolarised cellular membrane potentials, such as those during myocardial ischaemia, Na^+ fast channels are mostly in an inactivated state, which is why lidocaine's action is effective under these conditions (Balser*, et al.*, 1996; Xiao*, et al.*, 2004). Lidocaine has been shown to be effective in a range of animal studies during acute myocardial ischaemia (Borer*, et al.*, 1976; Spear*, et al.*, 1972), and in human patients with myocardial infarction (Koster & Dunning, 1985; Singh & Kocot, 1976).

From the evidence in the 1980s, lidocaine was recommended as the first-choice of anti-arrhythmic drugs in resuscitation guidelines (Kudenchuk & Racht, 1999). However, because of a lack of prospective randomised clinical trial data showing a benefit of lidocaine in cardiac arrest patients (Dorian*, et al.*, 2002; Somberg*, et al.*, 2002), it has since been relegated to a second-line drug after amiodarone (Neumar*, et al.*, 2010). Lidocaine has limited success to convert both shock-resistant VF and non-shockable rhythm in OHCA patients (Dorian*, et al.*, 2002; Herlitz*, et al.*, 1997; Singh, 2002) and this may relate to a higher defibrillation energy threshold required to convert VF to sinus rhythm (Dorian*, et al.*, 1986; Echt*, et al.*, 1989). However, two large multicentre studies showed unexpected benefits of lidocaine compared to amiodarone to improve resuscitation outcome in-hospital arrest (Pollak*, et al.*, 2006; Rea*, et al.*, 2006). The authors argued that the benefit may relate to the relatively short period of arrest in the in-hospital environment compared to longer out-of hospital arrest times that appear to favour the use of amiodarone (Rea*, et al.*, 2006). Recently, an 889 patient study also showed lidocaine was more beneficial in younger patients (<18 years old) than amiodarone (Valdes*, et al.*, 2014). Valdes et al. (2013) showed for children with in-hospital VT/VF, lidocaine, but not amiodarone, use was independently associated with improved ROSC and 24 hr survival, although neither drug was correlated with survival to hospital discharge. In 2013, prophylactic use of lidocaine upon ROSC was found to be beneficial to reduce recurrent arrhythmias (Kudenchuk*, et al.*, 2013). As a result of these newer studies, a growing consensus is emerging for lidocaine use to promote improved resuscitation outcomes, particularly for in-hospital cardiac arrest patients, which would be best addressed through prospective randomised controlled trials (Kudenchuk*, et al.*, 2013; Pollak*, et al.*, 2006).

1.8.2.2 Lidocaine as a cardioprotectant

Although less established in humans, lidocaine is found to provide cardioprotection and reduces infarct size in ischaemic myocardium in animal models (Boudoulas*, et al.*, 1978; Imaizumi*, et al.*, 2012; Kaczmarek*, et al.*, 2009; Kompa & Summers, 2000; Lesnefsky*, et al.*, 1989). In 2011, a randomised, double-blinded trial showed that intraoperative lidocaine infusion significantly reduced myocardial injury after off-pump coronary artery surgery (Lee*, et al.*, 2011). Lidocaine's cardioprotective effects include improved myocardial dysfunction, less apoptosis and reduced infarct size (Kaczmarek*, et al.*, 2009).

The mechanism underlying lidocaine cardioprotection is related to lidocaine-induced Na^+ channel blockage (Wang*, et al.*, 2007). Cell injury during ischaemia involves cell Ca^{2+} and Na^+ overloading leading to mitochondrial dysfunction and cardiac functional loss (Saris*, et al.*, 1993; Yasutake & Avkiran, 1995). By blocking Na^+ channels, lidocaine prevents Na^+-induced intracellular Ca^{2+} overload (Ebel*, et al.*, 2001), limits high energy phosphate waste due to reduced activity of the energy consuming Na^+/K^+-ATPase (Lee*, et al.*, 2011; Schaefer*, et al.*, 1994), and reduced mitochondrial ROS production (Iwai*, et al.*, 2002a; Iwai*, et al.*, 2002b). In the rat model of VF cardiac arrest, Wang and colleagues (2007) demonstrated that limiting sarcolemmal Na^+ entry using Na^+ channel inhibitors: 1) prevented left ventricular intracellular Na^+ loading, 2) prevented mitochondrial Ca^{2+} overloading during and after resuscitation, 3) maintained left ventricular compliance during chest compression, and 4) improved myocardial function post resuscitation (Wang*, et al.*, 2007).

1.8.2.3 Lidocaine as an anti-inflammatory agent and anti-oxidant

Lidocaine has anti-inflammatory properties and can down-regulate the immune functions of several cell lineages (Lahat*, et al.*, 2008; Yardeni*, et al.*, 2009). Lidocaine has been shown to inhibit neutrophil activation and endothelium adhesion molecules (ICAM-1) expression (Lan*, et al.*, 2004), impede monocyte chemoattractant protein (MCP-1) release (Li*, et al.*, 2003) and halt granulocyte adherence (MacGregor*, et al.*, 1980). More recently, lidocaine has been shown to attenuate interferon-γ and TNF-α release from human T cells (Lahat, 2008), and reduce COX-2 expression on inflamed mucosa in an ischaemia-injured jejenum model (Cook*, et al.*, 2009). Lidocaine attenuation on cytokine-induced cellular injury has been shown to mediate endothelial

and vascular smooth muscle protection, which is important to maintain vascular function and haemostasis (de Klaver*, et al.*, 2003).

Lidocaine also has potent free-radical scavenging (hydroxyl radical scavenger and singlet oxygen) (Gunaydin & Demiryurek, 2001; Lenfant*, et al.*, 2004; Tang*, et al.*, 2009) and anti-thrombolytic restorative properties (Huang*, et al.*, 2009). Das and colleagues suggested that the protective effect of lidocaine on myocardial injury may, in part, be due to its reactive oxygen scavenging properties (Das & Misra, 1992). Furthermore, it has been shown that lidocaine suppressed superoxide production from activated neutrophils in a dose-dependent manner, and this suppression correlated strongly with the suppression of translocation of p47 phox, a new subunit of NADPH-oxidase (Arakawa*, et al.*, 2001). In 2009, Tang and colleagues also showed that lidocaine reduced haemolysis of red blood cells by directly scavenging free radicals (Tang*, et al.*, 2009).

The anti-inflammatory and free radical scavenging effects of lidocaine are non-Na^+ fast channel dependent (Hollmann*, et al.*, 2001). They may involve lidocaine's interaction with specific K^+ channels (de Klaver*, et al.*, 2003), Ca^{2+} channels (Tanaka*, et al.*, 2002), membrane-associated enzymes (Cassuto*, et al.*, 2006), or G proteins (Hollmann & Durieux, 2000). To summarise, from lidocaine's anti-ischaemic, anti-inflammatory and ant-oxidant effects, the drug may be useful for cardiac arrest studies in the post-resuscitation phase to reduce ischaemia-reperfusion injury (Demaison*, et al.*, 2013; Wang*, et al.*, 2007).

1.8.3 Adenosine-lidocaine (AL) combination as a potential candidate for cardiac arrest treatment

1.8.3.1 The role of a "hibernating-like" state in cardiac surgery

In 1998, G.P. Dobson borrowed from the tricks of natural hibernators and introduced the concept of a pharmacologically induced 'hibernating-like' state using the combination of adenosine and lidocaine (AL) as a surgical cardioplegia solution (Dobson, 2004, 2010; Dobson*, et al.*, 2013). The objective was to induce pharmacological arrest at the resting 'polarised' membrane potential by blocking the Na^+ fast current channels responsible for depolarization particularly at phase O upstroke (lidocaine-armside of AL), while concomitantly activating K^+_{ATP} channels opening (with adenosine) to reduce the action potential duration (Dobson*, et al.*, 2013).

Natural hibernators do not dramatically reduce their cell's oxygen requirements by over 95% by depolarising the cells' membranes using high K^+, as is standard of practice in cardiac surgery today (Dobson, 2004).

The major benefits of maintaining the cell's membrane potential at or near resting state are: 1) reduced energy-dependent activity leading to better energy preservation during ischaemia (Dobson, 2004); 2) less electrolyte and metabolic imbalance, especially Na^+ and Ca^{2+} loading, potentially minimising cellular injuries (Cohen*, et al.*, 1995; Snabaitis*, et al.*, 1997); 3) decreased production of toxic metabolites (ROS, lactate, ion H^+) (Boutilier, 2001); and 4) anti-inflammatory effect from reducing neutrophil attachment and entry (Dobson, 2010). Since that time, there has been much preclinical work showing AL superiority in rats and dogs (Corvera*, et al.*, 2005; Dobson, 2010), and the use of AL cardioplegia in paediatric cardiac surgery has been shown to significantly improve myocardial function with reduced troponin I levels post operatively (Jin*, et al.*, 2008). Further, Onorati and colleagues showed in a randomised clinical trial that AL with magnesium and insulin in emergency cardiac surgery patients led to significantly improved myocardial protection, improved cardiac index and ventricular-arterial coupling, lower troponin, lower plasma lactate, a 50% reduction in the use of blood products, and two days less in the ICU compared to patients who received the conventional Buckberg solution (Onorati*, et al.*, 2013).

1.8.3.2 AL as an anti-arrhythmic agent

Canyon and Dobson showed in the rat model of 30 min regional myocardial ischaemia that over 50% of the animals died from arrhythmias (Canyon & Dobson, 2004). In direct contrast, infusion of AL five minutes before and during ischaemia led to no deaths and a 90% reduction of ventricular arrhythmias (Canyon & Dobson, 2004). Importantly, adenosine and lidocaine alone failed to prevent VF following myocardial ischaemia and reperfusion (Canyon & Dobson, 2004). It was the combination of adenosine and lidocaine that had potent anti-arrhythmic properties. The cardiac rhythm stabilisation with AL is possibly associated with defending the membrane potential closer to normal than controls, especially around the ischaemic border-zone, and thereby reducing abnormal excitability and reentry arrhythmias (Canyon & Dobson, 2004; Dobson, 2010).

1.8.3.3 AL as a cardioprotectant

AL has been shown to offer cardioprotection against ischaemia-reperfusion injury in both animal and human studies (Corvera*, et al.*, 2005; Dobson, 2010; Dobson & Jones, 2004; Jin*, et al.*, 2008; O'Rullian*, et al.*, 2008; Sloots & Dobson, 2010). Consistent with few or no arrhythmias, AL administration significantly improved survival and reduced myocardial infarct size in rats subjected to acute myocardial ischaemia (Canyon & Dobson, 2004). Again, this benefit was only observed with AL combination treatment and not with either drug alone (Canyon & Dobson, 2004). In addition, if the A_1 agonist CCPA was used instead of adenosine and combined with lidocaine the infarct size fell to below 10%, similar to preconditioning (Canyon & Dobson, 2005). Using ^{31}P NMR, Dobson and colleagues showed during coronary artery ligation myocardial protection was related to preservation of left ventricular high-energy phosphate (PCr and ATP) during ischaemia, suggesting a downregulation of myocardial metabolism with AL (Canyon & Dobson, 2006). Furthermore, the rate-pressure product (RPP) was significantly lower with AL treatment, indicating a lower myocardial oxygen demand (Canyon & Dobson, 2006). By lowering oxygen and energy demand during oxygen deprivation, AL appears to be reducing the pathological sequelae of ischaemia/reperfusion injury (Dobson*, et al.*, 2013).

Furthermore, AL cardioprotection has extended to organ preservation where isolated rat hearts immersed in AL recovered 80% of full cardiac function after six to eight hours of cold static storage (Rudd & Dobson, 2011a, 2011b). Moreover, there was no circulating troponin C detected and the lactate level was significantly lower in AL-preserved hearts, indicating reduced ischaemic damage regardless of the prolonged cold static storage (Rudd & Dobson, 2011b). The current safe period for human heart storage is four to five hours.

1.8.3.4 AL as an anti-inflammatory agent

In addition to lowering energy demand, the anti-ischaemic properties of AL appear to involve blunting of the inflammatory response. An *in vitro* study using activated pig neutrophils showed that AL inhibited neutrophil activation to a greater extent than adenosine or lidocaine alone (Shi*, et al.*, 2012). The anti-inflammatory effects with AL were shown to include inhibition of superoxide generation, adhesion molecule expression, neutrophil adherence and transmigration. Supporting this, the potent anti-

inflammatory action of AL has also been observed in an *in vivo* model of VF-induced cardiac arrest (Granfeldt*, et al.*, 2013).

1.8.3.5 Cardiac rescue and stabilisation with AL and ALM (AL with Mg^{2+}): translation from cardiac surgery to resuscitation science

In 2008, Dobson and colleagues hypothesised that the cardioprotective effect of AL *at non-arrest concentrations* may help to resuscitate the heart during shock and low-perfusion trauma states (Dobson, 2010). Recently, AL's potential use for resuscitation has been demonstrated in low-flow haemorrhagic shock models (Granfeldt*, et al.*, 2012; Letson & Dobson, 2011a, 2011b; Letson*, et al.*, 2012). It is shown that 7.5% NaCl/AL with Mg^{2+} (ALM) bolus following 40 to 60% blood loss and 60 min shock resulted in 100% survival and higher MAP with few or no arrhythmias compared to saline controls (Letson & Dobson, 2011a, 2011b). ALM cardiac resuscitation in rats following 40% blood loss generated higher arterial systolic and improved diastolic pressures, suggesting improved systemic vascular resistance (Letson*, et al.*, 2012). Letson and Dobson also showed when AL was combined with magnesium (ALM) the combination fully corrected acute traumatic coagulopathy after 60 min of hypotensive resuscitation (Letson*, et al.*, 2012). The mechanisms of correction are not known at present but may find great utility in cardiac arrest studies.

Furthermore, small-volume hypertonic AL or ALM resuscitation fluid has translated into the pig (Granfeldt*, et al.*, 2014; Granfeldt*, et al.*, 2012), and it was shown that a single IV bolus (4 ml/kg) increased MAP into the hypotensive range and increased stroke volume by a factor of two for 60 min before shed whole blood was returned (Granfeldt*, et al.*, 2014). A higher stroke volume occurred at a lower heart rate which was associated with a longer systolic LV ejection time (duration), a result that the authors argued allowed more blood to be ejected per beat compared to controls (Granfeldt*, et al.*, 2014). In addition, the superior haemodynamics with AL and/or ALM following shock in pigs was achieved simultaneously with reduced whole body oxygen consumption, improved oxygen delivery, reduced blood lactate, improved kidney function, and systemic protection (Granfeldt*, et al.*, 2014; Granfeldt*, et al.*, 2012).

Unlike the haemorrhagic shock model, there is limited data on the effect of AL in experimental cardiac arrest models. In 2013, Granfeldt and colleagues reported that after seven minutes of VF-induced cardiac arrest in pigs, AL led to lower end diastolic pressures (EDP) with higher left ventricular dP/dt_{max} and dP/dt_{min} compared to controls,

suggesting improved myocardial contractility and significantly improved protection against inflammation than controls (Granfeldt*, et al.*, 2013). However, there was no significant difference in neurohistological score of AL-treated animals compared to that of controls after 24 hours post VF-induced cardiac arrest (Granfeldt*, et al.*, 2013). It would be interesting to examine if the neurological effect of AL would be different when applied during resuscitation without epinephrine or after asphyxial-induced cardiac arrest.

1.9 AL with magnesium sulphate ($MgSO_4$): potential combination for resuscitation drug

Magnesium (Mg^{2+}) was added to AL in cardiac surgery for further electrical and metabolic stabilisation and to reduce Ca^{2+} entry into the cells for longer-term cross-clamp operations in adult and paediatric patients. Mg^{2+} is nature's natural calcium antagonist (Iseri & French, 1984). Mg^{2+} is also an important cofactor for cellular metabolism, including ATPases that have central roles in cell biochemistry (Champeil*, et al.*, 1983). Intracellular Mg^{2+} is also important in regulating protein and nucleic acid synthesis, cell cycle, mitochondrial integrity, ion transport, and signal transduction (Saris*, et al.*, 2000). Since Mg^{2+} plays important roles in modulating potassium (K^+) and calcium (Ca^{2+}) transport and signalling, its normal concentration is essential to preserve membrane stability (Saris*, et al.*, 2000) especially for muscular, neurological and cardiovascular physiological functions (Grubbs & Maguire, 1986; Mubagwa*, et al.*, 2007).

Mg^{2+} has well-known electrophysiological functions including action potential stimulation affecting most excitable cells including cardiac myocytes. Consequently, Mg^{2+} concentration has important consequences for myocardial functions, i.e. cardiac conduction and contractility (Agus & Agus, 2001). Extracellular Mg^{2+} provides direct inhibitory effects on Ca^{2+} channels (Hess, 1986), and partial effects on inward rectifier K^+ channels (Biermans*, et al.*, 1987) leading to increased action potential duration and thus negative chronotropic effects (Headrick*, et al.*, 1998). These inhibitory effects are augmented along with increased concentration of Mg^{2+} (up to 8.0 mM) (Headrick*, et al.*, 1998); yet, evidence of moderately higher Mg^{2+} concentration leading to action potential shortening is available (Zhang*, et al.*, 1995).

Apart from reducing heart rate (chronotropic negative), increased extracellular Mg^{2+}also leads to decreased contractility (inotropic negative), which is reversible with

Ca^{2+} administration (Headrick*, et al.*, 1998). The net result of increased Mg^{2+} is a reduction in myocardial oxygen consumption (Headrick*, et al.*, 1998). It is suggested that the Mg^{2+} negative inotropic effects might be achieved through direct inhibition of Ca^{2+} fluxes into sarcolemma (Shine & Douglas, 1974; White & Hartzell, 1988), or by indirect modulation of intracellular Mg^{2+} concentration, which in turn, inhibits sarcoplasmic reticulum Ca^{2+} (Hall & Fry, 1992). However, the later mechanism was not confirmed in Headrick and colleagues' (1998) study. Alternatively, Mg^{2+} may competitively bind to contractile proteins at the same site for Ca^{2+} binding preventing Ca^{2+}-induced contraction (Koss & Grubbs, 1994).

Magnesium has a long history in both cardiac surgery and resuscitation science. Experimental and clinical trials have investigated the potential of Mg^{2+} therapy during resuscitation, although, the results are inconclusive (Reis*, et al.*, 2008). In the 1990s, two different case studies reported successful resuscitation with intact neurological function after >1 hr intractable VF or asystole with Mg^{2+} treatment (Craddock*, et al.*, 1991; Tobey*, et al.*, 1992). Following this, a clinical study reported a higher rate of ROSC was achieved with Mg^{2+} treatment combined with standard ACLS protocol (Miller*, et al.*, 1995). In contrast, the administration of Mg^{2+} during and following resuscitation appeared to have no significant effect on ROSC attainment and survival to hospital discharge compare to placebo in in-hospital (Thel*, et al.*, 1997) or out-of-hospital cardiac arrest with VF rhythm (Allegra*, et al.*, 2001; Fatovich*, et al.*, 1997). Therefore, it will be interesting to investigate the resuscitation effects of magnesium combined with AL solution on cardiac arrest in an animal model.

1.10 General aim and hypotheses

The overall aim of this thesis is to investigate AL and ALM's protective role in treating cardiac arrest following 8 min asphyxial hypoxia in the rat *in vivo*.

The following hypotheses will be tested:

1. AL will improve haemodynamics and ECG stabilisation during intermittent chest compressions following asphyxial cardiac arrest in rats.

2. AL cardioprotection will further be improved with induced hypothermia during and after cardiac arrest, manifesting as increased ROSC achievement and sustainability post-cardiac arrest.

3. AL combined with magnesium sulphate (ALM) will further enhance cardiac function during hypothermia, and result in improved post-ROSC haemodynamics despite low temperature (28-33^{o}C).

4. The combined therapies (ALM and intra-arrest hypothermia) will reduce early post-cardiac arrest syndrome, including cardiac deterioration and haemodynamic instability, and correct coagulopathy and possibly reduce brain injury.

5. ALM with intra-arrest hypothermia will improve resuscitation outcomes and post-resuscitation haemodynamics compared to standard-of-care epinephrine.

CHAPTER 2. MATERIALS AND METHODS

2.1 Introduction

This chapter describes in detail the animal housing conditions, ethics, methods of anaesthesia, chemical preparations, experimental set-up, surgical protocols and the asphyxial cardiac arrest model and measurement endpoints. Details of experimental design and statistical analysis and the number of treatment groups are not included here but outlined in the following chapters since as each study has different experimental design.

2.2. Animal and chemical preparation

Male Sprague-Dawley rats (300-450 g) were obtained from James Cook University breeding colony. The rats were fed *ad libitum* with free access to water and housed in a 12-hr light-dark cycle (Ethics approval number A1540 and A1910). Non-heparinised animals were anaesthetised with an intra-peritoneal injection of 100mg/kg sodium thiopentone (Thiobarb, Lyppards, Queensland). Thiobarb was administered as required throughout the protocol. All studies in this thesis conformed with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Adenosine (A9251), magnesium sulphate and other chemicals were obtained from Sigma Chemical Company (Castle Hill, NSW). Lidocaine hydrochloride was purchased as a 2% solution (ilium) from Lyppards, Queensland.

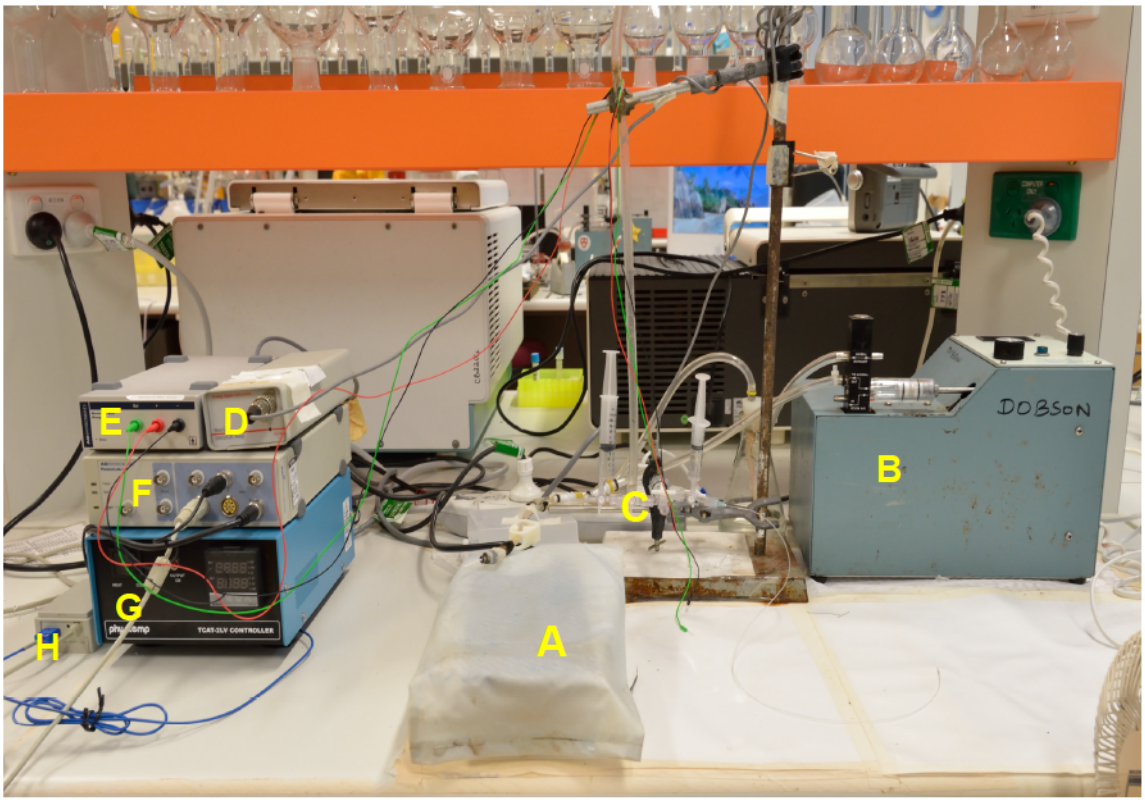


Figure 2.1 Photograph of the *in vivo* asphyxial cardiac arrest model set up
A) rat cradle with warm blanket, B) rodent ventilator, C) Blood pressure transducer set up, D) MacLab BridgeAmp (Blood pressure measurement), E) MacLab BioAmp (Heart rate measurement) with ECG leads, F) ADInstruments Powerlab, G) Temperature controller, H) Rectal probe

2.3 Surgical protocol

The experimental set up is depicted in Figure 2.1. Anesthetised animals were positioned in the supine position on a custom designed cradle. A tracheotomy was performed and animals were artificially ventilated (95-100 strokes min^{-1}) on humidified room air using a Harvard Small Animal Ventilator (Harvard Apparatus, Mass., USA). The left femoral vein and artery were cannulated using PE-50 tubing for drug infusions and blood pressure monitoring (UFI 1050 BP coupled to a MacLab BridgeAmp). Lead II electrocardiogram (ECG) leads were implanted subcutaneously on the left and right front legs and earthed on the back leg (see Fig. 2.2). Heart rate was obtained from these ECG leads using a small animal biological amplifier (ML-136) coupled to a Powerlab. The arterial blood pressures and heart rate were recorded using Chart 5 software (ADInstruments, Sydney, Australia). A rectal probe was inserted 5.0 cm from the rectal orifice for temperature measurements.

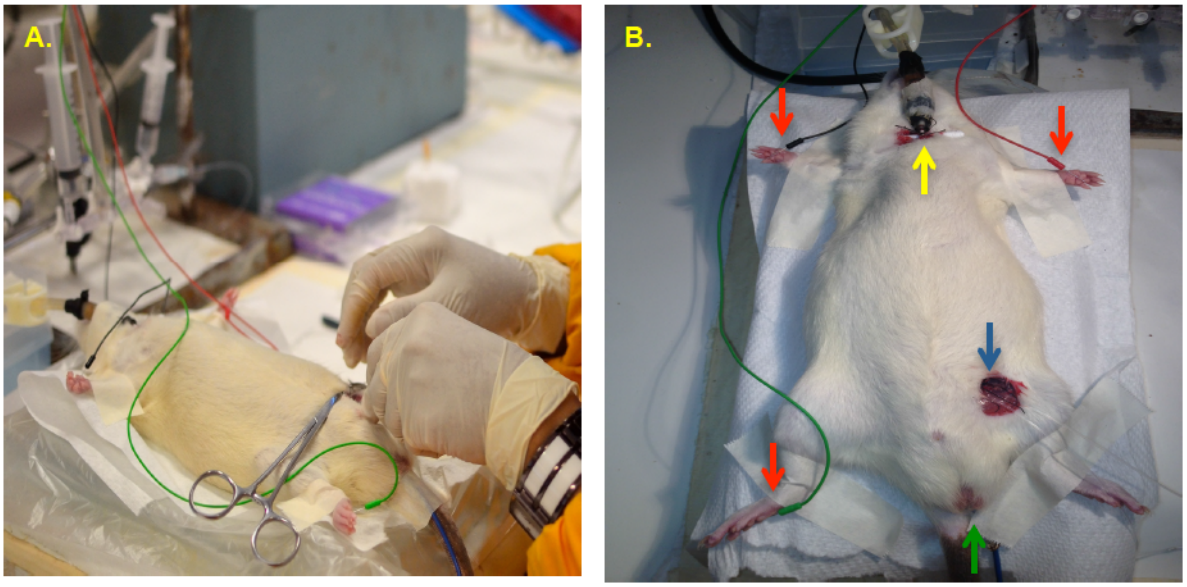


Figure 2.2 Photographs of the surgical protocols for rat model of asphyxial cardiac arrest
(A) Cannulation of left femoral artery and vein, (B) Anesthezised rat in supine position with tracheal intubation (yellow arrow), femoral artery/vein cannulation (blue), ECG lead (red) and rectal probe (green) placement

2.4 Asphyxial-induced cardiac arrest model

Asphyxial cardiac arrest in the rat was induced by turning off the ventilator and clamping the tracheotomy tube from the ventilator for eight minutes. Before inducing cardiac arrest, rats were stabilized for 10-15 minutes to assess the baseline haemodynamics. Cardiac arrest was defined when the mean arterial pressure was less than 10 mmHg (MAP <10mHg). Turning off the ventilator and clamping the tracheotomy tube resulted in bradycardia and eventually pulseless electrical activity within 3-4 min, which progressed to asystole in less than 5% of the animals after 6-7 min. Pilot studies found that animals with asystole are more difficult to resuscitate and required prolonged chest compressions with large variability in outcomes. Therefore, for this thesis animals that progressed to asystole were excluded to ensure a similar cardiac arrest profile for all controls and treatment groups.

2.4.1 Resuscitation attempt

Resuscitation was attempted in three steps:

1) administering an IV bolus of 0.5 ml drugs through the femoral vein,10 seconds prior to chest compressions
2) turning the ventilator back on and restarting positive mechanical ventilation (95-100 strokes min^{-1}), and
3) commencement of chest compressions.

Chest compressions was performed manually using the index and middle fingers and compressing down on the rat chest wall about 30% of the anterior-posterior *diameter* of the chest (~1 cm compression depth with full recoil) at the rate of 300 compressions per minute (McCaul*, et al.*, 2009). The rate of compressions was confirmed using a MacLab system (see section 2.4.3 Haemodynamic measurements). Chest compressions were re-applied for up to three cycles if no return of spontaneous circulation (ROSC) was achieved (SAP <20 mm Hg). No other treatments including vasopressors were applied post-resuscitation.

2.4.2 Intermittent chest compression

In the first two studies (Chapters 3 and 4), intermittent chest compressions were employed to study haemodynamic rescue and stabilization during each compression phase. The first set of chest compressions was applied for 60 sec and repeated for 30 second (up to three times) if no ROSC was achieved within the first 5 min window after turning on the ventilator. In pilot studies, the established ROSC was non-sustainable and ceased within 5 min, therefore, a further set of 30 sec chest compressions were administered, and this sequence was repeated every 5 min for 60 min to observe the haemodynamic rescue and stabilization during chest compressions as well as hands-off periods over time.

2.4.3 Induced hypothermia

Before turning off the ventilator, surface cooling was initiated by placing an ice-cold pack under the animal, and ethanol spray was used intermittently during 8-min asphyxial hypoxia. A rectal probe was inserted 5.0 cm from the orifice to measure the temperature. Lomax (2011) reported that this method provided temperatures approximately 0.5^{o}C lower than core temperature. With the surface cooling in the pilot

studies, the rectal temperature fell from 36-37°C at baseline to ~33°C prior to resuscitation. Surface cooling was either continued post-resuscitation to induce moderate hypothermia (32-28^{o}C) or removed and the animal actively rewarmed with a heating lamp (0.02-0.06^{o}C/min) immediately after ROSC depending on experimental design in each study.

2.4.4 Haemodynamic measurements

Heart rate (HR; beats.min^{-1}), systolic pressure (SAP; mmHg), diastolic pressure (DAP; mmHg) and mean arterial pressure (MAP; mmHg) were monitored throughout baseline, cardiac arrest and post-ROSC period. HR was obtained from the Lead II ECG using a small animal biological amplifier (ML-136) and three-lead Bio Amp cable coupled to a MacLab and Chart 5.5 software for continuous recording, visualization and analysis (ADInstruments, Sydney, Australia) (see Fig. 2.3). The arterial blood pressure recording was used to visually and quantitatively validate the ECG-derived heart rate measurements. The two methods were compared using Chart 5.5 software, and agreement provided assurance to accurately capture the heart rate from the ECG during cardiac arrest and resuscitation. MAP is generally thought of as an index of perfusion pressure of the vital organs and tissues where MAP = (2/3 x diastolic pressure) + (1/3 x systolic pressure), or diastolic pressure + 1/3 (systolic - diastolic pressure). Episodes and duration of arrhythmias were recorded using lead II ECG and identified using the Lambeth convention as previously outlined in Canyon and Dobson study (2004). Ventricular fibrillation (VF) was defined as QRS deflections that could not easily be distinguished and HR could not be measured, and ventricular tachycardia (VT) was defined as four or more consecutive ventricular premature beats (premature QRS complexes).

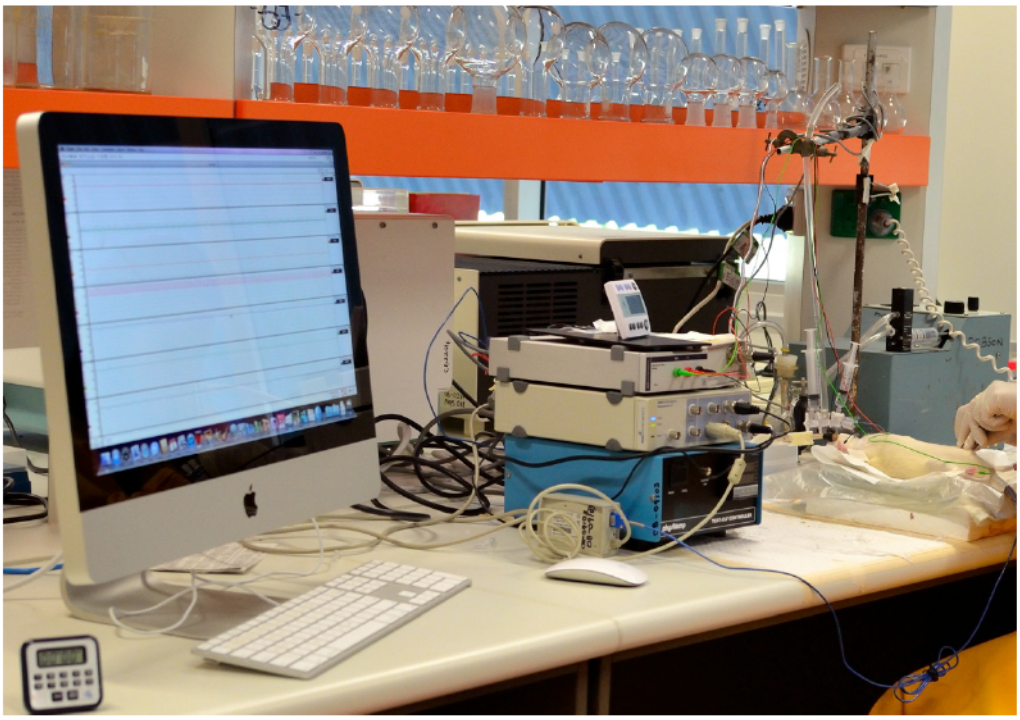


Figure 2.3 The Maclab data acquisition system
Heart rate and blood pressures (MAP, SAP, DAP) were continuously monitored using three-lead BioAmp and BridgeAmp, respectively, coupled to a MacLab and Chart 5.5 software for continuous recording, visualization and analysis.

2.5 Assessment of Coagulopathy

Coagulation measurements in this thesis comprise at least one of two coagulation analyses:

1) **Conventional Plasmatic Methods**

   Conventional coagulometry was used to analyse prothrombin time (PT) and activated partial thromboplastin time (aPTT). PT is a parameter of extrinsic and final (common) pathway clotting time, while aPTT is a parameter of intrinsic and common pathway clotting time. Clinically, these standard coagulation tests are used to assess a patient's response to blood thinners (e.g. warfarin), as they reflect the kinetics of first fibrin formation expressed as clotting time, in seconds. The contributions of platelets cannot be assessed since the PT and aPTT tests use plasma only.

   Measurement protocols for PT and aPTT analysis: At the end of the experiment, venous whole blood was withdrawn from the femoral vein into a 2 ml vacutainer containing citrate-phosphate-dextrose solution (0.14/mL) to chelate

Ca^{2+} and prevent activation of the coagulation system. Additional groups of rats were assigned to baseline and cardiac arrest groups, and 2 ml blood samples were withdrawn during the baseline and cardiac arrest states. Animals were sacrificed after blood withdrawal.

Blood samples were centrifuged (Hettich, Universal 16R) at room temperature (25°C) at 3000 rpm for 15 min (Fig. 2.4A). The plasma was removed, snap frozen in liquid nitrogen, and stored at −80°C until use. PT and aPTT were assessed using a microcoagulometer (Amelung KC4Delta; Trinity Biotech, Ireland) (Fig. 2.4B). Fifty microlitres of plasma was placed in a rotated tube containing a steel ball. PT was measured by adding 100 µL of thromboplastin reagent containing $CaCl_2$ (TriniCLOT PT; Trinity Biotech) in the pre-incubated (1 min at 37°C) plasma; while aPTT was measured by incubating 50 µL plasma with 50 µL aPTT regent (TriniCLOT aPTT; Trinity Biotech) followed by the addition of 50 µL of $CaCl_2$ solution. Clotting times were recorded when the fibrin clot started to form and the steel ball stopped rotating. All measurements were performed in triplicate.

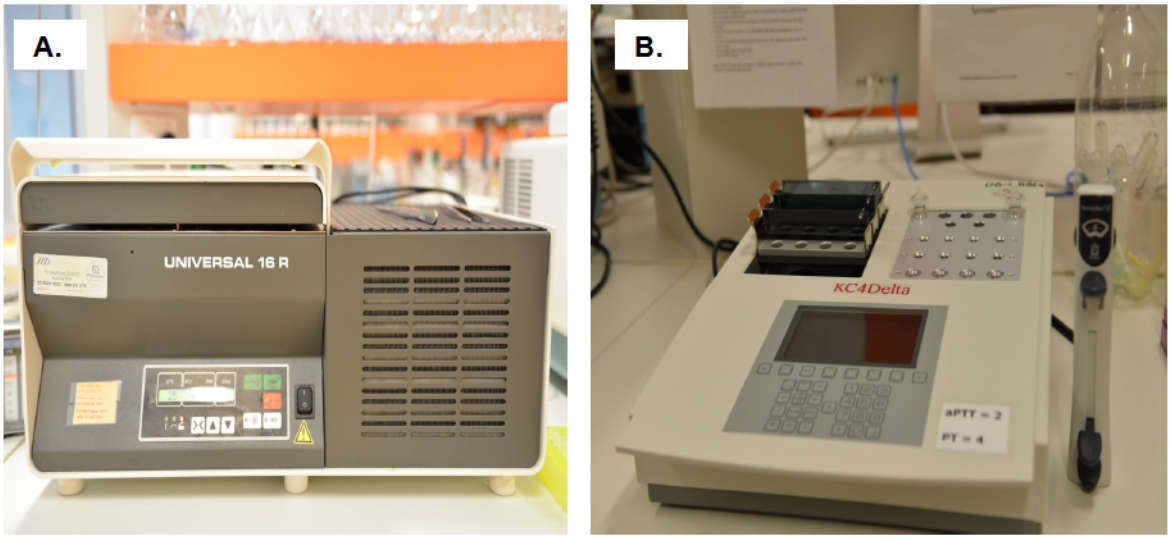


Figure 2.4 Photographs of devices used for PT and aPTT measurement
(A) Centrifuge Universal 16R and (B) microcoagulometer Amelung KC4Delta

2) Rotational thromboelastometry (ROTEM)

Rotational thromboelastometry (ROTEM, Tem International, Munich Germany) (Fig. 2.5A) provides a real-time evaluation of the viscoelastic properties of whole blood from initiation of the clot, early formation kinetics, firmness and prolongation, fibrin-

platelet interactions and clot lysis (Lang, *et al.*, 2005). This whole blood coagulation analysis more closely reflects the dynamics of haemostasis *in vivo*.

Measurement protocols for ROTEM analysis: Venous whole blood was obtained at baseline, and at 120 min following ROSC or, in those animals that failed to attain ROSC, in the first 2-5 min of attempts. Blood was drawn into a 2.0 ml BD vacutainer containing citrate-phosphate-dextrose solution to chelate Ca^{2+} and prevent activation of the coagulation system (Letson, *et al.*, 2012). Baseline and cardiac arrest blood samples were withdrawal from additional groups of rats to avoid alteration of coagulation due to hypovolemic or diluted blood.

Within 30 min of blood withdrawal, blood was warmed at 37°C for 5-10 min, and EXTEM, INTEM and FIBTEM viscoelastic analysis was performed. The EXTEM test is extrinsically activated by thromboplastin (tissue factor) and EXTEM CT is a measure of integrity of the extrinsic and final common coagulation pathways analogous to PT. In contrast, the INTEM test, activated by the contact phase, is a measure of the integrity of the intrinsic and final common pathways analogous to aPTT. The FIBTEM test is activated as in EXTEM with the addition of cytochalasin D, which inhibits platelet glycoprotein (GP) IIb/IIIa receptors. The FIBTEM test thus provides information about the effect of fibrin polymerization on clot strength and is independent of platelet involvement.

The following parameters were measured: Clotting time (CT), the time from start of measurement until a clot amplitude of 2 mm; clot formation time (CFT), the time from end of CT until a clot firmness of 20 mm; and maximum clot firmness (MCF), the final strength of the clot in mm arising from the interaction of fibrin and activated by platelets and factor XIII. The alpha angle (α) was also measured and represents the angle between baseline and a tangent at the maximum clot slope and clot amplitude (amplitude at 5 to 30 min) in mm over a 30 min period. The lysis index (LI, %) was estimated as the ratio of clot firmness (amplitude at 30 or 60 min) divided by MCF multiplied by 100. LI is an estimate of fibrinolysis, and hyperfibrinolysis was defined as estimated percent lysis ≥15% at 60 min (Lang, *et al.*, 2005). Maximum clot elasticity (MCE) was calculated from MCE = (MCF x 100)/(100 - MCF) (Lang, *et al.*, 2009).

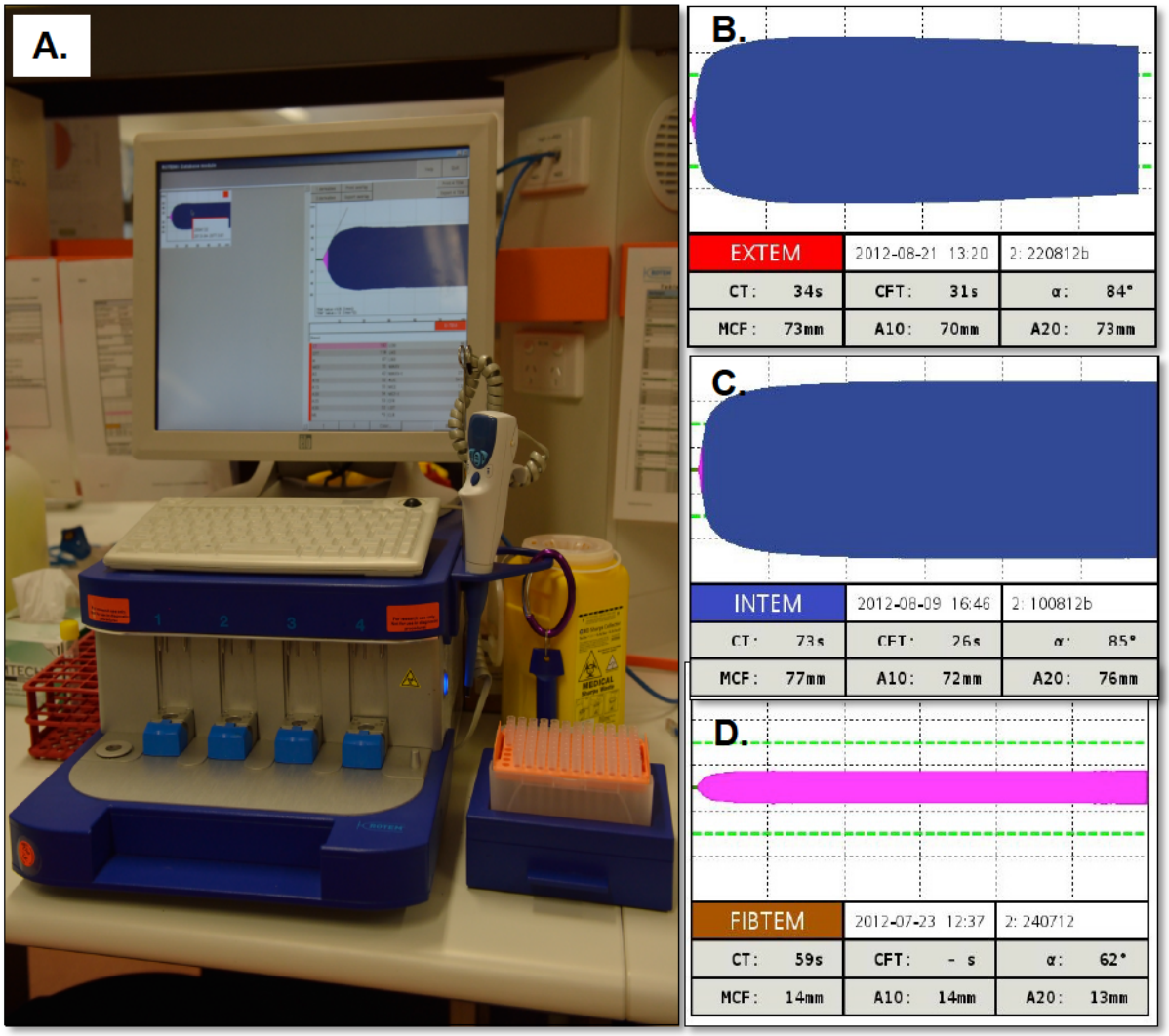


Figure 2.5 Photographs of rotational thromboelastometry ROTEM
(A) ROTEM apparatus and ROTEM trace obtained from EXTEM (B), INTEM (C) and FIBTEM (D) tests of whole blood from healthy rats

2.6 Neurological Study

Neurological assessment was performed in Chapter 6 to examine the effects of adenosine, lidocaine and magnesium (ALM) on early neurological changes (2 hr post ROSC) compared to saline controls following 8 min asphyxial cardiac arrest. The assessment was carried as followed.

2.6.1 Neuronal damage quantification

Rats were decapitated at the end of the observation period. The brains were carefully removed, post fixed in 10% formaldehyde for at least three days and embedded in paraffin. Coronal sections 5-µm thick were serially sliced with a microtome and stained with haematoxylin and eosin (H & E). Two subsequent sections (Bregma -3.3) of each brain sample were examined by an investigator blinded to the treatment groups using a microscope (Nikon eclipse 50i) and photographed with digital sight camera (Fig. 2.6) and NIS-elements software.

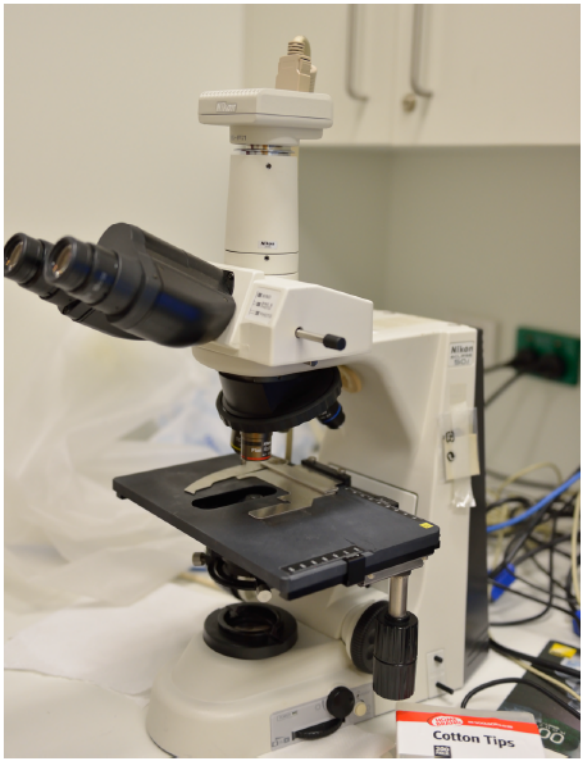


Figure 2.6 Photographs of microscope (Nikon eclipse 50i) coupled with digital sight camera

The number of injured neurons was quantified in two different areas:
1) ***Hippocampal CA1***. Ischaemic neurons were bilaterally counted by direct visualisation at high magnification (400x) along the medial to lateral axis. The CA1 is chosen as this area is selectively vulnerable to cerebral ischaemia in animals including rats (Jia, *et al.*, 2008).
2) ***Neocortex***. This area was captured at 100x total magnification to cover a broader area of cortex. Quantitative analysis of ischaemic neurons was performed manually in both hemispheres using ImageJ cell counter software (National Institutes of Health, USA) to avoid miscounting. A previous study observed the presence of neurological changes within hours of ischaemic insult in the cortical region (Yamamoto, *et al.*, 1999).

The number of ischaemic neurons presented for each rat is the average of the total number of damaged neurons in both hemispheres (either CA1 or cortical region) in two subsequent sections. Three additional rats underwent surgical protocol without asphyxiation and were sacrificed as sham controls.

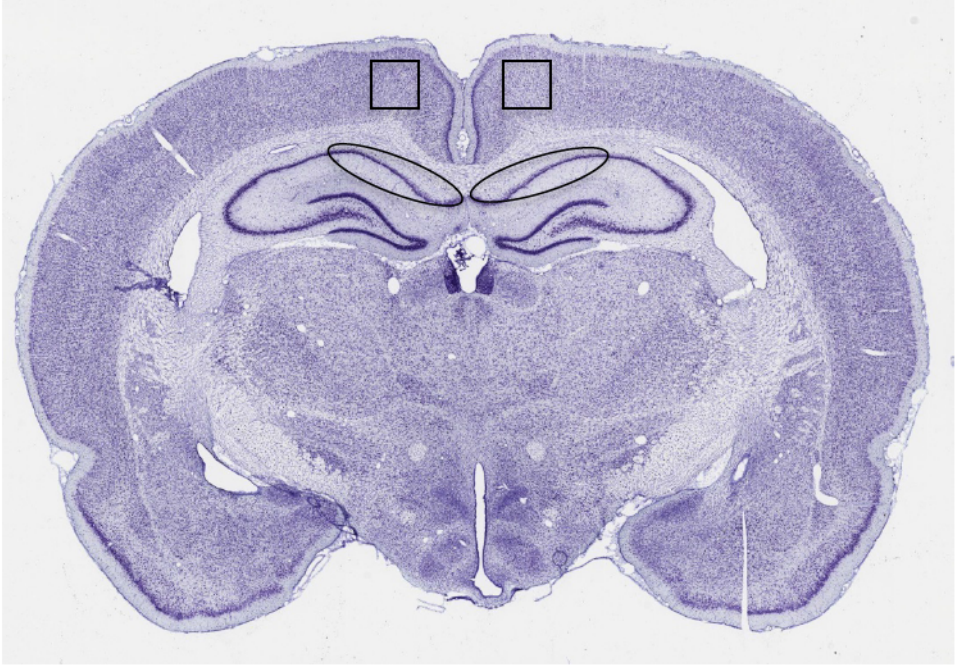


Figure 2.7 Illustration of rat brain coronal section at bregma -3.3 utilized for ischaemic neuron quantification
The squared areas represent the neocortex, while the oval areas depict hippocampal CA1.

2.6.2 Category of damaged neurons

Ischaemic neurons were identified by criteria previously described by Yamamoto, *et al.* (1999), including dark shrunken morphology, strong eosinophilic, pyknotic nuclei, and irregularly distributed cytoplasm. Only neurons that show no recognizable nucleus or low-nuclear-cytoplasmic border were counted (see Fig. 2.8). These categories describe the most injured neurons (type IV and type III) found in cerebral ischaemic rats (Eke, *et al.*, 1990).

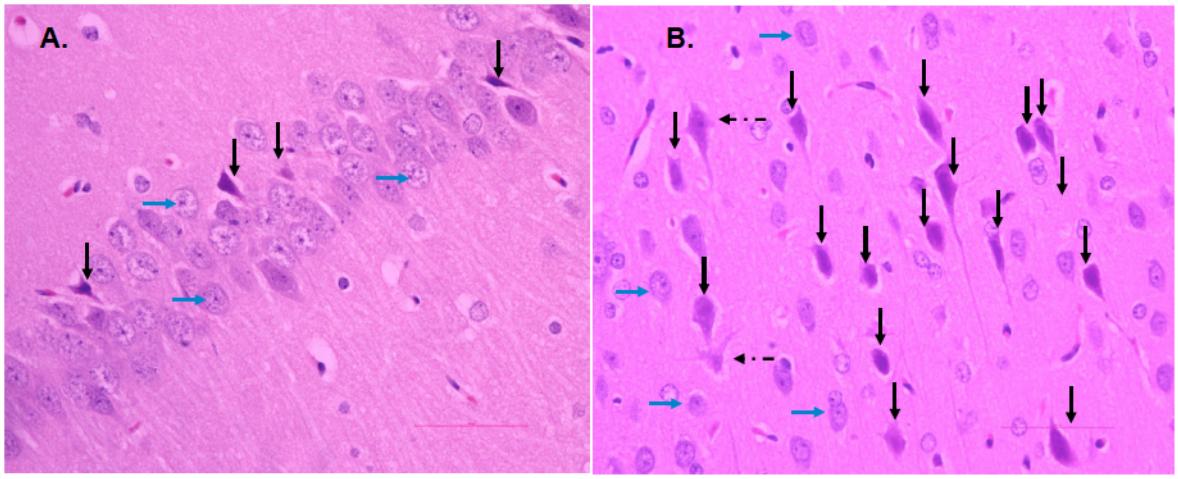


Figure 2.8 Ischaemic neurons in hippocampal CA1 (A) and cortical region (B) at 400x magnification
Eosinophilic cytoplasm and shrunken morphology distinguishes ischaemic from normal neurons (blue arrow). Only neurons with indistinctive nucleus (black solid arrow) or low-nuclear-cytoplasmic contrast (black dashed arrow) were counted.

CHAPTER 3. THE EFFECT OF ADENOSINE AND LIDOCAINE (AL) COMBINATION, OR ADENOSINE AND LIDOCAINE ALONE, ON HAEMODYNAMIC RESCUE AND ECG STABILITY DURING CHEST COMPRESSIONS AFTER 8 MIN ASPHYXIAL HYPOXIA IN THE RAT[1]

3.1 Introduction

Asphxial hypoxia-induced cardiac arrest is associated with bradycardia, pulseless electrical activity (PEA) and asystole (Engdahl*, et al.*, 2003a; Ornato & Peberdy, 1996) and has very poor outcomes (<10% survival) (Hallstrom*, et al.*, 2007; McCaul*, et al.*, 2006a). Poor prognosis in pre-hospital and in-hospital cardiac arrest arises from the current inability of resuscitation and drug therapies to rescue and stabilise the heart and brain (Berg*, et al.*, 2010; Charalampopoulos & Nikolaou, 2011; Ewy & Sanders, 2013; Olasveengen*, et al.*, 2009b). The aim of this chapter was to investigate the effect of adenosine (A), lidocaine (L), and AL combined to rescue and stabilise the heart and haemodynamics during intermittent chest compressions during accidental hypothermia.

3.2 Aims

1. To determine the optimal dose for AL intravenous injection to improve haemodynamic and electrocardiogram (ECG) stability during chest compressions following 8 min asphyxial hypoxia in rats.
2. To compare the effect of AL, adenosine, and lidocaine alone on haemodynamic and ECG stability during chest compressions following 8 min asphyxial hypoxia in rats. Animals were not actively warmed to maintain body temperature.

[1]Study 2 of Chapter 3 has been published in *American Journal of Emergency Medicine.* 2013 Nov;31(11):1539-1545 (see detail on page vii)

3.3 Experimental Protocols

3.3.1 Drug preparation and experimental groups

Study 1. AL dose response study:

Stock solutions containing 9 mM adenosine and 18 mM lidocaine HCl were prepared on the day of the experiment. AL stock concentration was chosen based on a preliminary study using 6 min asphyxial cardiac arrest in rats (unpublished data). AL injections were made in 5 different doses with 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml of stock solution, which contained adenosine/lidocaine: 0.24/0.5 mg; 0.48/1.0 mg; 0.72/1.5 mg; 0.96/2.0 mg; and1.2/2.5 mg, respectively. The volume of bolus injection was made up to 0.5 ml (in 0.9% saline) for all doses. The study consisted of five experimental groups (n=8 per group): 1) AL 0.1 ml, 2) AL 0.2 ml, 3) AL 0.3 ml, 4) AL 0.4 ml, and 5) AL 0.5 ml.

Study 2. The effect of optimal dose of AL, adenosine, or lidocaine alone on resuscitation:

The concentrations of adenosine (0.48 mg), lidocaine (1.0 mg), and AL (0.48/1.0 mg) were prepared in 0.5 ml 0.9% saline on the day of experiment. The concentrations were chosen based on the optimal concentration of AL from the dose-response study (Study 1). The study consisted of four experimental groups (n=8 per group): (1) 0.9% saline (SAL), (2) adenosine (ADO), (3) lidocaine (LIDO), and (4) adenosine/lidocaine (AL).

3.3.2 Experimental design

Animal housing, feeding and water regimes, ethics approvals, methods of anaesthesia and surgical protocols are described in Chapter 2. Male Sprague-Dawley rats (300-400 g) were assigned to one of the experimental groups as described above. Each group consisted of eight rats. The method of inducing asphyxial cardiac arrest has been described in detail in Chapter 2. Briefly, cardiac arrest was induced by turning “off” the ventilator, and clamping the tracheotomy tube for 8 min. Immediately after removing the clamp and turning “on” the ventilator, a 0.5 mL bolus of drug or saline was injected intravenously (IV) followed by 60 sec of chest compressions (300/min). After 60 sec of chest compressions, animals were observed for ROSC. If ROSC was not achieved or when systolic pressure (SAP) fell below 20 mmHg, 30 sec compressions were applied.

ROSC was considered unachievable in the first 5 min window if it could not be attained after three attempts of chest compressions after turning the ventilator back on (See Fig 3.1). After 5 min, a second set of 30 sec compressions was administered, and this sequence was repeated every 5 min for 60 min. Preliminary studies showed that, in this model, no ROSC was achieved after 5 min in any group. Thus, the protocol was designed to study ROSC in the first 5 min and haemodynamic rescue and stabilisation *during each compression phase* every 5 min, for a total time of one hour (see Fig. 3.1). Heart rate, SAP, DAP, and MAP were monitored throughout the study.

Definitions: ROSC was defined as a SAP >20 mmHg. Cardiac arrest was defined as a MAP <10 mmHg (Fig. 3.1). Survival was defined as those animals that achieved ROSC. Episodes and duration of arrhythmias were recorded using lead II ECG and identified using the Lambeth convention, as previously outlined in Canyon and Dobson (Canyon & Dobson, 2004). Ventricular fibrillation (VF) was defined as QRS deflections that could not easily be distinguished and as HR that could not be measured, and ventricular tachycardia (VT) was defined as four or more consecutive ventricular premature beats (premature QRS complexes).

IV Bolus Treatment (injection dose)

STUDY 1:

- 0.5 ml AL (adenosine and lidocaine 0.24/0.5 mg)
- 0.5 ml AL (adenosine and lidocaine 0.48/1.0 mg)
- 0.5 ml AL (adenosine and lidocaine 0.72/1.5 mg)
- 0.5 ml AL (adenosine and lidocaine 0.96/2.0 mg)
- 0.5 ml AL (adenosine and lidocaine 1.2/2.5 mg)

STUDY 2:

- 0.5 ml 0.9% NaCl
- 0.5 ml adenosine (0.48 mg)
- 0.5 ml lidocaine (1.0 mg)
- 0.5 ml AL (adenosine and lidocaine 0.48/1.0 mg)

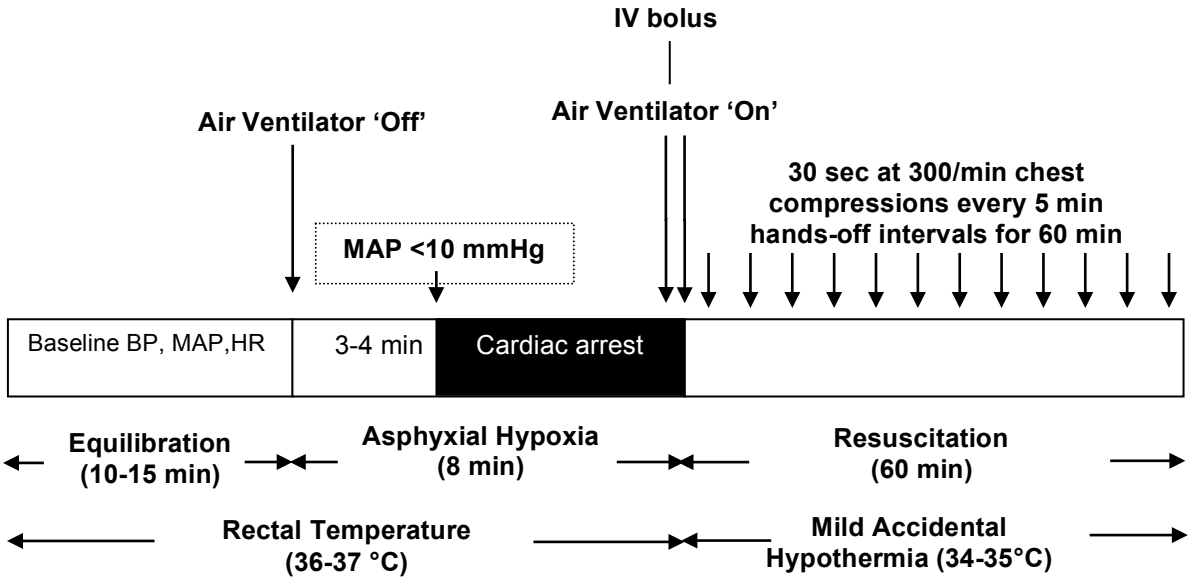


Figure 3.1 A schematic of the *in vivo* asphyxial hypoxia model to induce cardiac arrest in the rat

Cardiac arrest (MAP <10 mmHg) occurred within 3-4 min after turning off the ventilator. After turning the ventilator back on, 0.5 mL treatment bolus was injected IV and immediately followed by 60 seconds of chest compressions. After 5 min hands-off period, another 30 seconds of chest compressions was administered, and this 5 min sequence was repeated for 60 minutes. Animals were not actively warmed to maintain body temperature.

3.3.3 Statistical Analysis

A priori power analysis was performed using G-power program for analysis of variance (ANOVA), repeated measures between factors, based on the number of treatment groups and 12 measurements to a 95% confidence interval. A sample size of eight generated an absolute power (1- β probability error) of 1.0. All values were expressed as mean ± SEM. Data distribution was tested with *Kolmogorov-Smirnov* for normality. Systolic pressure, DAP, MAP, and HR were evaluated using a one-way ANOVA at specific time points, followed by a *Tukey*'s post hoc test. Two-way ANOVA comparison was used to evaluate haemodynamic changes within a group. The number of animals that achieved ROSC and the incidence and duration of arrhythmias (in seconds) were compared using a *Mann-Whitney U* test. Statistical significance was defined as a $p<0.05$.

3.4 Results

STUDY 1: Dose response AL and optimal resuscitation

3.4.1 AL dose response study

3.4.1.1 Haemodynamic data at baseline and during asphyxia

The baseline haemodynamic data (SAP, DAP, MAP, and HR) was not significantly different between groups (Table 3.1). Immediately after clamping the ventilator tube, blood pressure and HR increased but within 30-40 seconds the MAP dropped significantly. After 1-2 min there was another increase in MAP before it eventually fell below 10 mmHg, which was defined as cardiac arrest (Fig 3.2). The average time from the initiation of asphyxia to cardiac arrest for all animals was approximately 4 min, and was not significantly different between groups (Table 3.1).

Table 3.1 Comparison of baseline haemodynamic data, time to cardiac arrest (CA) and CA duration among AL groups

Group	n	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (bpm)	Time to CA (sec)	Duration of CA (sec)
0.1 ml	8	119 ± 3.1	108 ± 3.5	113 ± 2.9	314 ± 11.1	232 ± 9.7	247 ± 9.7
0.2 ml	8	121 ± 4.7	95 ± 5.9	103 ± 4.5	305 ± 12.7	241 ± 10.7	238 ± 10.7
0.3 ml	8	107 ± 3.1	93 ± 1.8	97 ± 2.1	280 ± 8.2	238 ± 11.4	242 ± 11.4
0.4 ml	8	116 ± 5.7	100 ± 7.5	106 ± 6.5	304 ± 7.9	237 ± 8.0	243 ± 8.0
0.5 ml	8	121 ± 5.1	110 ± 5.2	115 ± 5.3	316 ± 7.8	248 ± 6.6	231 ± 6.6

Values are expressed as mean ± SEM. SAP = systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, HR = heart rate, Time to CA = time to cardiac arrest, from the initiation of asphyxia to the onset of cardiac arrest (MAP <10 mmHg), Duration of CA = duration of cardiac arrest. No significant difference found between groups.

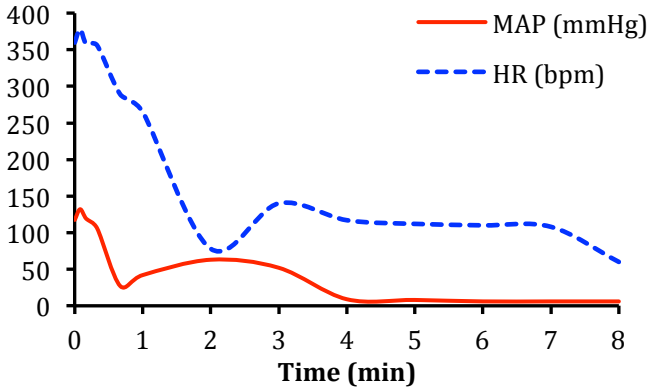


Figure 3.2 Representative changes in mean arterial pressure (MAP) and heart rate (HR) during 8 min asphyxial-induced cardiac arrest

3.4.1.2 Effect of increasing AL dose on ROSC achievement following 8-min asphyxia

Following AL injection and 60 sec chest compressions, all rats receiving the lower dose range (0.1 ml, 0.2 ml, 0.3 ml) achieved ROSC, compared to 50% of rats that received the 0.4 ml bolus and 82.5% that received the 0.5 ml AL bolus (see Table 3.2). The duration of ROSC was variable in all treatment groups and ranged from 31±13.1 to 161±70.2 sec, with the lower dose range showing longer ROSC durations than the higher dose range (0.4 to 0.5 ml) (Table 3.2).

Table 3.2 Effect of different AL doses on ROSC achievement and duration

Group (n=8)	Animal achieving ROSC	Duration of ROSC (sec)
0.1 ml	8 (100%)	119 ± 34.6
0.2 ml	8 (100%)	161 ± 70.2
0.3 ml	8 (100%)	140 ± 27.8
0.4 ml	4 (50%)	31 ± 13.1
0.5 ml	7 (82.5%)	59 ± 14.1

Values are expressed as mean ± SEM. ROSC achieved when SAP >20mmHg during hands-off period. Duration of ROSC =the period from ROSC achievement to the time when SAP <20 mmHg.

3.4.1.3 Haemodynamics during chest compressions

The effect of different AL doses on the return of developed pressures, SAP, DAP and MAP are shown in Fig 3.3.

Systolic Arterial Pressure (SAP): The highest to lowest pressures (in mmHg) at the time of the first set of compressions (t = 0 sec) were 0.2 ml (54 ± 4.0), 0.3 ml (54 ± 4.8), 0.1 ml (48 ± 3.3), 0.5 ml (38 ± 2.7) and 0.4 ml AL (28 ± 4.2). SAP in the 0.2 ml group remained >20 mmHg during the first 30 min and >15 mmHg at 60 min. All other doses were lower with the significant differences outlined in Fig 3.3.

Diastolic Arterial Pressure (DAP): The highest to lowest pressures (in mmHg) at the time of the first set of compressions (t = 0 sec) were 0.1 ml (33 ± 2.6), 0.2 ml (27 ± 1.3), 0.5 ml (26 ± 2.0), 0.3 ml (24 ± 1.4) and 0.4 ml AL (19 ± 2.7). At 30 min DAP of 0.2 ml AL-treated rats was 17 ± 1.9 mmHg during compressions which was significantly higher compared to both 0.1 ml and 0.4 ml dose groups up to 60 min. At 60 min, only 0.2 ml group rats continued to produce DAP >10 mmHg with chest compressions. It is noteworthy that the highest diastolic pressure at t=0 with 0.1 ml AL was not lasting and dropped by 75% to less than 10 mmHg at 30 min which was significantly lower than 0.2 ml AL developed diastolic arterial pressure.

Mean Arterial Pressure (MAP): The highest to lowest pressures (in mmHg) at the time of the first set of compressions (t=0) were 0.2 ml (45 ± 2.7), 0.3 ml (43 ± 3.5), 0.1 ml (43 ± 3.0), 0.5 ml (34 ± 2.4) and 0.4 ml AL (25 ± 3.6). MAP for the 0.1 ml AL group and 0.3 ml groups drastically decreased to <10 mmHg and <15 mmHg, respectively after 30 min, whereas 0.2 ml AL-treated rats defended their MAPs at around 20 mmHg. At 60 min, the 0.2 ml AL group maintained compression-produced MAP of ~15 mmHg, while all other groups had MAPs below 10 mmHg. The MAP in the 0.2 ml group was significantly higher than the 0.4 ml group throughout resuscitation, and the 0.1 ml group after 25 minutes (see Fig 3.3).

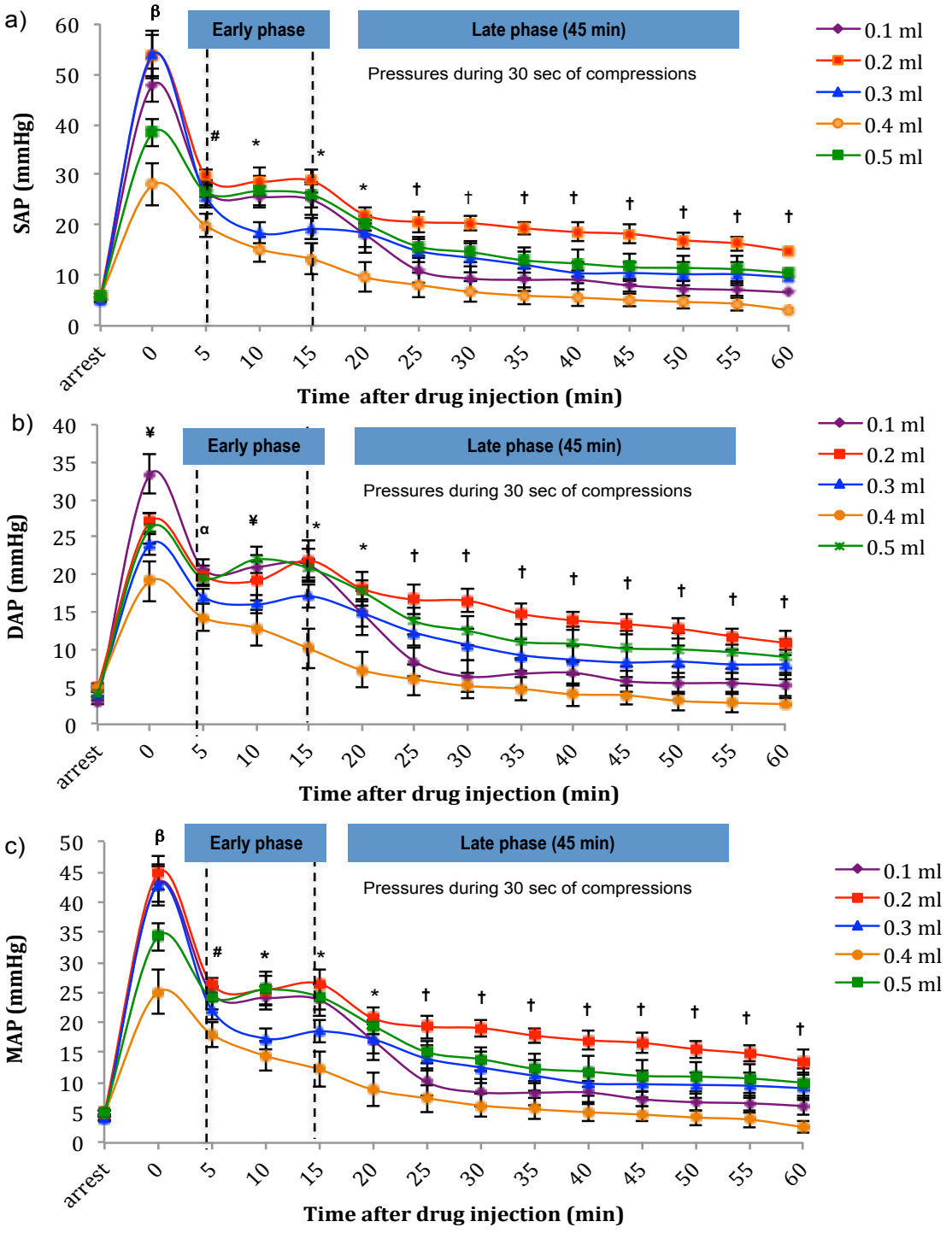


Figure 3.3 a) Systolic arterial pressure (SAP), b) diastolic arterial pressure (DAP), c) mean arterial pressure (MAP) during 30-sec compressions separated by 5-min hands-off periods over 60 min

Values are expressed as mean ± SEM. $^{\beta}p<0.05$ for 0.1 ml, 0.2 ml, 0.3 ml compared to 0.4 ml group. $^{\#}p<0.05$ for 0.2 ml compared to 0.4 ml group. *p<0.05 for 0.2 ml and 0.5 ml compared to 0.4 ml group. $^{\dagger}p<0.05$ for 0.2 ml compared to 0.4 ml and 0.1 ml groups. $^{¥}p<0.05$ for 0.1, 0.2, 0.5 ml groups compared to 0.4 ml group.

3.4.1.4 Cardiac (ECG) stabilisation

Only four rats out of 40 AL-treated animals experienced arrhythmias over the range of doses, but none of them developed VF (Table 3.3). Arrhythmias occurred in only one animal in each group up to 0.4 ml. All VT events were self-correcting and animals converted to sinus rhythm, and these mostly occurred during chest compressions. One animal injected with 0.1 ml AL showed frequent VT (9 in number), but the total duration was less than 8 sec (non-sustainable VT). Meanwhile, the longest duration of VT was found in the 0.4 ml group, with one animal experiencing a VT event lasting for more than 60 seconds.

Table 3.3 Frequency and duration of arrhythmias found in animals treated with different doses of AL

Group	Number of animals experienced arrhythmias		Frequency of arrhythmias per animal		Duration of arrhythmias per animal (sec)	
	VT	VF	VT	VF	VT	VF
0.1 ml AL	1	0	9	0	7.5	-
0.2 ml AL	1	0	2	0	4	-
0.3 ml AL	1	0	4	0	5	-
0.4 ml AL	1	0	2	0	61.5	-
0.5 ml AL	0	0	0	0	-	-

Values are expressed as mean ± SEM. VT = ventricular tachycardia, VF = ventricular fibrillation.

3.4.1.5 Choice of bolus AL concentration for future experiments

Based on the effect of increasing AL concentrations on ROSC and its duration, developed SAP, DAP and MAP and ECG stability, it was concluded that 0.2 ml AL was the most optimal for resuscitation. Thus, the amount of adenosine and lidocaine in the 0.2 ml bolus (0.48 mg adenosine and 1.0 mg lidocaine) was used for all future experiments for this thesis and delivered in a total bolus volume of 0.5 ml 0.9% saline.

STUDY 2: The effect of optimal AL and adenosine (ADO) or lidocaine (LIDO) alone on resuscitation

3.4.2 Effect of AL (0.48mg/1.0 mg) compared to adenosine (0.48 mg) and lidocaine (1.0 mg) alone in 0.5 ml bolus on resuscitation after asphyxial cardiac arrest in the rat

3.4.2.1 Haemodynamic data at baseline and during asphyxia

The baseline haemodynamic data are shown in Table 3.4. No significant differences were found among the groups. Baseline arterial SAP ranged from 115 to 118 mmHg, DAP from 81 to 84 mmHg, MAP from 92 to 95 mmHg, and HR from 301 to 305 bpm. Times to cardiac arrest for the four groups were 4.2 to 4.5 min over the 8 min asphyxial hypoxia period.

Table 3.4 Comparison of baseline haemodynamic data, time to cardiac arrest (CA) and duration of CA among treatment groups

Group	n	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	Heart rate (bpm)	Time to CA (sec)	Duration of CA (sec)
SAL	8	115 ± 2.0	82 ± 3.0	93 ± 2.5	305 ± 8.7	252 ± 11.7	228 ±11.7
ADO	8	115 ± 3.0	81 ± 2.9	92 ± 2.7	301 ± 9.2	267 ± 6.0	213 ± 6.0
LIDO	8	118 ± 3.4	82 ± 2.8	94 ± 2.9	305 ± 7.6	253 ± 6.6	227 ± 6.6
AL	8	117 ± 4.6	84 ± 2.8	95 ± 2.8	304 ± 8.8	253 ± 6.3	227 ± 6.3

Values are expressed as mean ± SEM. SAL = saline, ADO = adenosine, LIDO = lidocaine, AL = adenosine-lidocaine. SAP = arterial systolic blood pressure, DAP = arterial diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, Time to CA = time to cardiac arrest from the initiation of asphyxia to the onset of cardiac arrest, Duration of CA = duration of cardiac arrest.

3.4.2.2 ROSC after 60 seconds of chest compressions

Five (62.5%) of the SAL animals, four (50%) of the ADO rats, seven (87.5%) of the LIDO rats, and 100% (eight) of the AL group achieved ROSC after drug injections and 60 sec chest compressions (Table 3.5). The number of AL rats that achieved ROSC was significantly higher than the ADO group (U =16.0, p =0.025). The total ROSC duration was not significantly different among the groups and ranged between 84 and 126 sec (Table 3.5). All animals, whether achieving ROSC or not, received intermittent chest compressions starting at 5 min after resuscitation was attempted, and are included in the data analysis.

Table 3.5 Effects of different treatment on ROSC achievement and duration following drug injection and 60-sec chest compression

Group	Animal achieving ROSC	Duration of ROSC (sec)
SAL	5 (62.5%)	84 ± 15.8
ADO	4 (50.0%)	109 ± 17.3
LIDO	7 (87.5%)	126 ± 23.9
AL	8 (100%)*	109 ± 12.4

Values are expressed as mean ± SEM. ROSC achieved when SAP >20mmHg during hands-off period. Duration of ROSC=the period from ROSC achievement to the time when SAP <20 mmHg. *p<0.05 compared to ADO group.

3.4.2.3 Haemodynamics during chest compressions

During the early phase of chest compressions (5-15 min), SAP was highest in the AL group, followed by the LIDO, ADO, and SAL groups (Fig. 3.4a). At 15 min, AL group SAP was significantly higher than ADO or SAL. During the later phase, the higher SAP for the AL group persisted at each compression interval for 60 minutes. Although SAP was up to ~24% higher in the AL group during the early compressions (26 mmHg vs 21 mmHg at 15 min) and up to 38% higher during the later phase (18 mmHg vs 13 mmHg at 55 min) compared to LIDO, these differences were not significant. Diastolic pressure in the AL group showed a similar profile to SAP in the early phase; however, in the later phase (35-45, and 55 min), it was significantly higher than any group (Fig 3.4b). From 15 to 60 min, DAP for the SAL group was 4-10 mmHg; for the ADO group, 4-12 mmHg; for the LIDO group, 7-12 mmHg; and for the AL group, 12-19 mmHg (Fig. 3.4b). Mean arterial pressure for the AL group was significantly higher than any group at 35, 40, and 55 min and higher than SAL and ADO groups between 20 and 60 min ($p<0.05$; Fig. 3.4c).

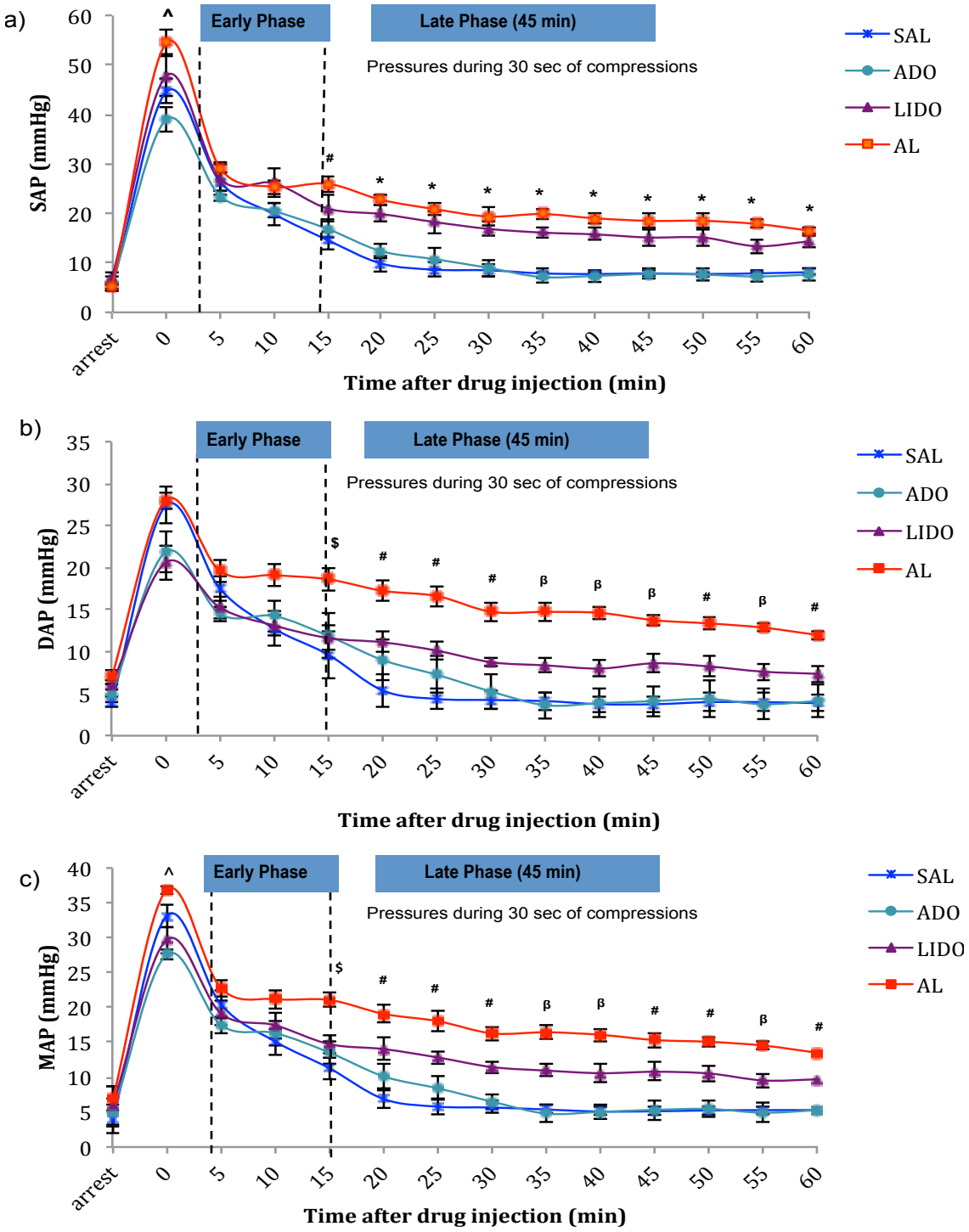


Figure 3.4 Arterial blood pressures during 30 sec of compressions separated by 5 min hands-off periods over 60 min

a) Systolic arterial pressure (SAP), b) diastolic arterial pressure (DAP), c) mean arterial pressure (MAP). Values represent the mean ± SEM. ^p<0.05 AL for higher SAP or MAP compared to ADO group (t=0). $^{\#}$p<0.05 for AL compared to ADO and SAL groups. *p<0.05 for AL and LIDO compared to ADO and SAL groups. $^{\beta}$p<0.05 for AL compared to all other groups. $^{\$}$p<0.05 for AL compared to SAL group. $^{\alpha}$p<0.05 for LIDO compared to SAL group. $^{\mu}$p<0.05 for LIDO compared to ADO and SAL groups.

3.4.2.4 Heart rates during hands-off periods

Spontaneous HR cannot be measured reliably during active chest compressions. Heart rate measured during the 12 hands-off periods is shown in Fig. 3.5. Heart rate in the AL group was up to 45% less than that in the SAL group and ~20% lower than LIDO or ADO groups throughout most of the hands-off period. The AL group was significantly lower than the SAL group at t=0 and during the first and fourth intervals (Fig. 3.5).

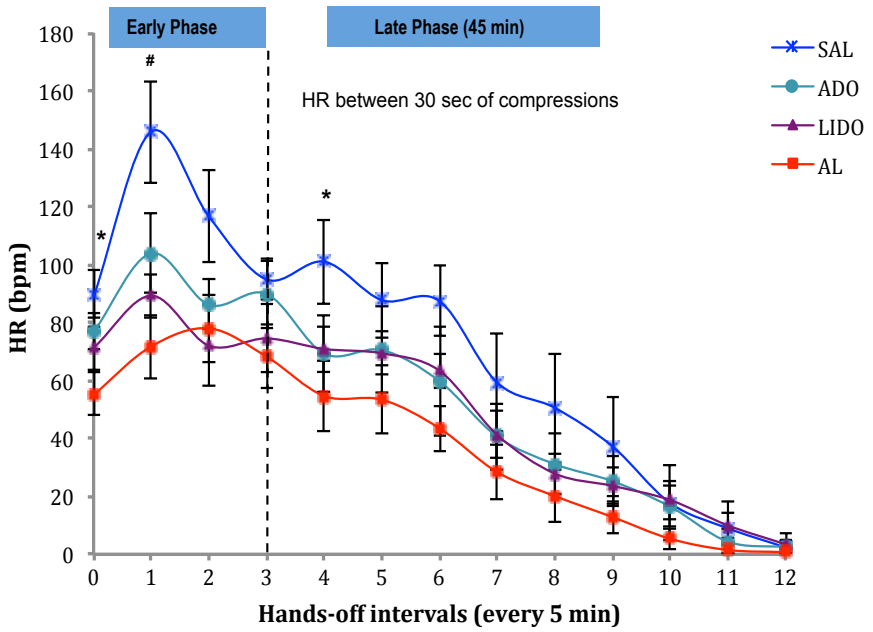


Figure 3.5 Heart rate measured during 5 min hands-off periods for 60 minutes
Each period ended with 30 sec chest compressions (see Materials and Methods). Values represent the mean ± SEM. *p<0.05 AL compared with SAL (t=0 and 4). #p<0.05 AL and LIDO groups compared with SAL.

3.4.2.5 Ventricular arrhythmias during hands-off intervals and chest compressions

The number of VT and VF that occurred during the early phase (i.e. first three hands-off periods) is shown in Table 3.6. Five of the eight SAL-treated rats experienced severe VT lasting 25 sec, and three of these had VF lasting 95 sec; four of eight ADO rats experienced VT lasting 20 sec and no VF; one LIDO rat experienced VF lasting 40 sec; and one AL rat experienced VT lasting 4 sec (Table 3.6). Representative ECG traces during the early hands-off periods are shown in Fig. 3.6A. Arrhythmias during the compression phase after the first 5 min are shown in Fig. 3.6B. A more stable

rhythm in the AL-treated rats was noted throughout, with small standard errors in R-R intervals compared with the other treatment groups (Fig. 3.6B).

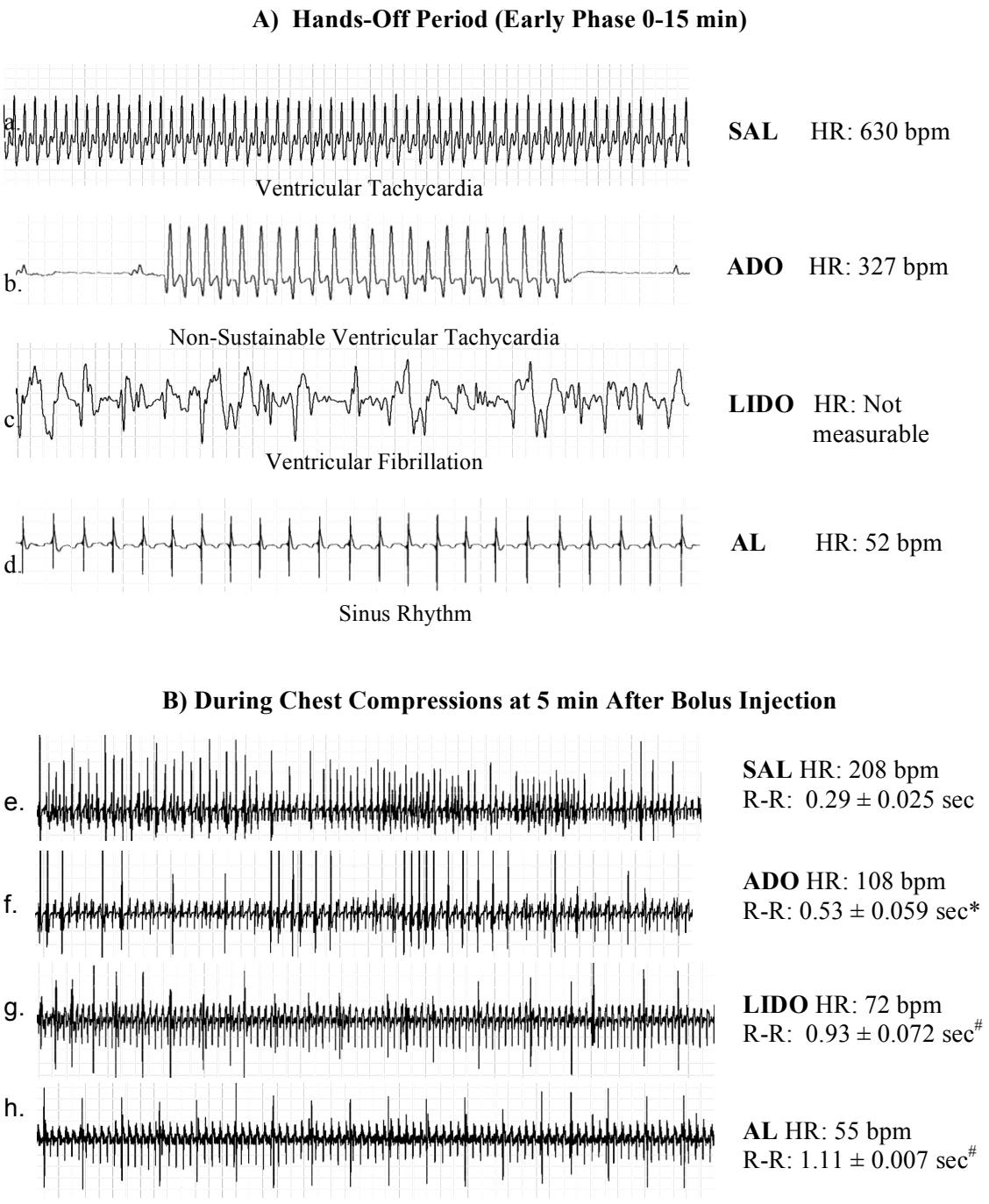


Figure 3.6 Representative traces of HR and rhythm among the four groups during the early hands-off period (A) and during the first 20 sec of chest compressions (B) 5 min after injection

The R-R interval was defined as the time elapsing between 2 consecutive R waves in the ECG measured over a period of 20 sec. Values represent the mean ± SEM. *p<0.01 for ADO compared with SAL group. #p<0.01 for AL and LIDO compared with ADO and SAL groups.

Table 3.6 Arrhythmic events with different treatments over the first 15 min in the hands-off period after 8 min asphyxial hypoxia

Group	Number of animals experiencing arrhythmias			Frequency of arrhythmias per animal			Duration of arrhythmias per animal (sec)		
	VT/VF	VT	VF	VT/VF	VT	VF	VT/VF	VT	VF
SAL	5	5	3	6 ± 1.4	5 ± 1.4	1 ± 0.3	82.4 ± 26.7	25.4 ± 6.6	95.0 ± 33.1
ADO	4	4	0	4 ± 0.4	4 ± 0.4	0	19.6 ± 8.6	19.6 ± 8.6	-
LIDO	1*	0	1	1*	0	1	40	-	40
AL	1*	1	0	2*	2	0	4	4	-

Frequency and total duration of VT expressed as mean ± SEM. SAL = saline, ADO = adenosine, LIDO = lidocaine, AL = adenosine-lidocaine.
VT/VF = ventricular tachycardia and/or fibrillation, VT = ventricular tachycardia, VF = ventricular fibrillation. *$p<0.05$ for AL and LIDO compared to SAL group.

3.5 Discussion

Currently, there is no rescue drug therapy that improves survival outcome after cardiac arrest in the adult or paediatric population (Nolan*, et al.*, 2002; Tress*, et al.*, 2010). This chapter showed that the optimal dose of AL to improve haemodynamic and cardiac stability during chest compressions after 8 min asphyxial hypoxia was 0.48/1.0 mg adenosine/lidocaine (0.2 ml group) in a total volume of 0.5 ml bolus. Using this dose, AL treatment led to improved haemodynamics (MAP, SAP, DAP) at lower HRs compared to either adenosine or lidocaine alone and saline controls during compressions, and resulted in a more stable ECG rhythm during the hands-off periods.

3.5.1 Improved haemodynamics during chest compressions with AL (0.48/1.0 mg)

High-quality chest compressions during cardiopulmonary resuscitation (CPR) are essential for buying time, however, at best, they can only provide 20 to 40% of cerebral blood flow and 10 to 20% of normal coronary blood flow, with almost no forward flow to the periphery (Gazmuri & Becker, 1997; Kern, 2000; Rubertsson & Karlsten, 2005; Yannopoulos*, et al.*, 2005). Increased coronary perfusion during chest compressions is vital for ROSC achievement (Kern, 2000; Paradis*, et al.*, 1990; Sutton*, et al.*, 2013). While this is mostly accomplished using vasopressor drugs during standard resuscitation, it may increase the risk of neurological damage and death during hospitalization (Hagihara*, et al.*, 2012; Olasveengen*, et al.*, 2012).

When compared to each drug alone, AL-treated rats developed higher SAP, DAP and MAP for lower HRs during chest compressions after asphyxial-induced cardiac arrest (Figs. 3.4 and 3.5). MAP during chest compressions was consistently higher in the AL-treated rats compared with all groups ($P<0.05$ at 35-45 min and 55 min) followed by the lidocaine group, and was lowest in adenosine and saline groups ($P<0.05$). SAP followed a similar pattern. In addition, DAP in the AL-treated rats was significantly higher from 25 to 60 min than lidocaine and adenosine alone or saline during chest compressions, and HR was 30-40% lower compared to controls over the 60 min experimental period.

Higher developed pressures in AL-treated animals may arise from: 1) a more compliant or distensible myocardium permitting more blood to enter its chambers during each decompression phase, 2) improved peripheral resistance leading to better developed pressures during compressions, and/or 3) improved coronary flow from higher diastolic arterial pressures. A more compliant heart is important for resuscitation because studies in experimental cardiac arrest have shown progressive reduction of left ventricular (LV) myocardial distensibility due to LV wall thickening during CPR (Ayoub*, et al.*, 2003; Gazmuri*, et al.*, 2008), which contributes to a progressive decline in haemodynamics during chest compressions (Klouche*, et al.*, 2002).

The second possibility for improved haemodynamics with AL is improved blood flow to the heart during compressions. The significantly higher DAPs in the AL group of 12 to 19 mmHg may improve blood flow to the globally ischaemic heart because the coronary perfusion pressure gradient is the difference between aortic diastolic and right atrial pressures (Kern, 2000; Reynolds*, et al.*, 2010). In addition, a higher DAP may reflect a higher peripheral vascular tone with improved forward flow to vital organs (Chamberlain*, et al.*, 2008; Kern, 2000). In the 1990s, Paradis and colleagues showed that a coronary perfusion pressure gradient of 15 mmHg or more was associated with improved ROSC (Paradis, 1996; Paradis*, et al.*, 1990). Rittenberger and colleagues (2006) also reported that patients with higher diastolic blood pressures after witnessed cardiac arrest had higher rates of ROSC during aeromedical transport. Recently, it has also been reported that ~1 mL/kg of 7.5% NaCl AL with Mg^{2+} led to a significantly higher DAP and improved MAP in the rat after 40% to 60% blood loss and 60 minute shock (Letson & Dobson, 2011a, 2011b). Further work is required to investigate the mechanism of improved haemodynamics with AL following 8 min of asphyxial hypoxia.

3.5.2 AL improved heart rhythm stability during chest compressions

Another interesting result from this chapter was a more stable ECG in the AL-treated animals during and between compressions compared to adenosine and lidocaine alone, as well as saline controls (Fig. 3.6A,B). Despite a few non-sustainable VT, there was no VF in AL-treated animals at any given dose (see Table 3.4). In contrast to AL, more arrhythmias (VT and/or VF) were encountered in the other groups (Table 3.7). A more stable ECG (rhythm and rate) with AL treatment was also reported by Canyon and Dobson (2004) in the rat model of myocardial infarction where the left

anterior descending coronary artery was tied off for 30 minutes. In the Canyon and Dobson study, AL treatment resulted in a 90% reduction in arrhythmias compared with adenosine and lidocaine alone (Canyon & Dobson, 2004).

Canyon and Dobson attributed the antiarrhythmic and anti-ischaemic properties of AL to improved electrophysiological stability in the highly arrhythmogenic border zones of ischaemic and non-ischaemic regions, and a down-regulation of metabolism of the left ventricle, as assessed by 31P nuclear magnetic resonance (Canyon & Dobson, 2006). In addition, dramatically fewer arrhythmias have also been reported during resuscitation after haemorrhagic shock (40% blood loss) in the rat using small-volume 7.5% NaCl AL with Mg^{2+} (Letson & Dobson, 2011a). Even in a near-lethal rat model of 60% blood loss and 80 minute shock, there were no ventricular arrhythmias during hypotensive resuscitation or during reinfusion of shed blood in the 7.5% NaCl AL/Mg^{2+} group compared with 7.5% saline controls (Letson & Dobson, 2011b). In the present study, a more compliant less stiff heart with AL therapy may also improve electrical stability because an increased incidence of arrhythmias has been associated with increased wall stress (Miura*, et al.*, 2012). Further studies are required to determine the antiarrhythmic effects of AL compared with ADO and LIDO alone in this asphyxial hypoxia model.

3.5.3 Potential clinical significance

The potential clinical significance of the results presented in this chapter may reside in assisting paramedics to treat out-of-hospital cardiac arrest patients who have not achieved ROSC. A bolus of AL IV administered as soon as possible on location followed by continuous mechanical chest compressions using a commercial "thumper" during transport to hospital may improve outcomes. Unlike the present rat protocol (see below limitations section), there must be minimal or no interruptions in chest compressions until arrival at the ICU where patients may be placed on a cardiopulmonary bypass machine (Berg*, et al.*, 2010).

3.5.4 Limitations of the study

The rat model used in this chapter and thesis has its limitations. The immediate administration of drugs, for example, is not a realistic clinical scenario because emergency dispatch protocols and paramedics take time to respond. In addition, the prolonged pauses between chest compressions in the present study are not clinically

relevant because it would severely limit ability to achieve ROSC. The current guidelines for CPR and emergency cardiovascular care strongly recommend that compressions be administered continuously with very brief intervals (seconds not minutes) to allow assessment of the patient, insertion of airway devices and lines, or changing rescuers (Berg*, et al.*, 2010; Bradley, 2011; Fox*, et al.*, 2013). Lastly, another possible limitation of this study may involve variability in depth and force of manual sternal compressions during each cycle. A continuous rat "thumper' with constant rate, force, and depth sensors may be more beneficial.

3.6 Conclusion

In this chapter, it was concluded that 0.48 ADO/1.0 mg LIDO (0.2 ml group) in a total volume of 0.5 ml bolus was most optimal compared to other AL doses for resuscitation in the 8 min asphyxial hypoxia rat model of cardiac arrest. Secondly, it was concluded that this AL bolus dose improved haemodynamics and ECG stabilisation during closed chest compressions compared with adenosine, lidocaine, or saline controls.

CHAPTER 4. EFFECT OF AL AND LIDOCAINE ALONE ON CARDIAC RESCUE AND STABILISATION DURING INTERMITTENT CHEST COMPRESSIONS AND A SINGLE SET OF COMPRESSIONS: EFFECT OF HYPOTHERMIA

4.1 Introduction

In Chapter 3, it was shown that a single bolus of AL improved diastolic pressures and cardiac stabilisation *during chest compressions* over a 60 min period compared to adenosine and lidocaine alone and saline controls in a rat model of asphyxial cardiac arrest. The data further showed that haemodynamic rescue was not optimal *because* ROSC could not be sustained for any group after 5 min resuscitation at body temperatures ranging between 34 to 35°C (accidental hypothermia). The aim of this chapter was to examine if induced therapeutic hypothermia applied during the intra-arrest period (33°C) and continued for 60 min resuscitation (28 to 32°C) improved the haemodynamic outcome in the rat model of asphyxial hypoxia.

4.2 Aims

The aims of the present Chapter were twofold:

1) To examine the effects of AL, L and saline controls following normothermic (36-37°C) and hypothermic (28-33^{o}C) resuscitation on ROSC, haemodynamics and cardiac stabilisation *during 13 sets of chest compressions* over 60 min following 8 min asphyxial hypoxia in rats. Adenosine was not included because of the high incidence of arrhythmias and high mortality in Chapter 3 (see 4.3.1 experimental design).
2) Some cold-arrested groups from Aim 1 showed that ROSC could be achieved after the first hands-off period and sustained for 60 min without further resuscitation. A second aim was to examine and compare ROSC attainment, haemodynamics and incidence of arrhythmias in the three cold groups after a single set of 60 sec of compressions. No further compressions were applied.

4.3 Experimental Protocols

4.3.1 Experimental design

Animal housing, feeding and water regimes, ethics approvals, methods of anaesthesia and surgical protocols are described in Chapter 2.

Study 1: Effects of AL, lidocaine and saline following normothermic and hypothermic intermittent chest compressions

Male Sprague-Dawley rats (300-400 g) were randomly assigned to one of six groups: 1) saline warm, 2) saline cold, 3) lidocaine warm, 4) lidocaine cold, 5) AL warm, and 6) AL cold. Each group consisted of eight animals. The cold groups were subjected to surface cooling (ice-cold pack and ethanol spray) applied during asphyxiation to target 33^{o}C at the time of resuscitation, and continued to 28^{o}C by the end of the 60 min observation period (see Fig. 4.1). An adenosine alone group was not included in this chapter because in the previous chapter animals treated with adenosine alone had a higher number of arrhythmias and poor survival (four out of eight failed to achieve ROSC).

Resuscitation protocol was the same as previously described in Chapter 3. Briefly, resuscitation was commenced by declamping the ventilation tube and injecting 0.5 ml bolus of drugs IV 10 sec before restarting the ventilator (at the same stroke rate of 95-100 strokes min^{-1}), followed by chest compressions (300/min) for 60 sec. After 60 sec of chest compressions, animals were observed for ROSC in the first 5 min hands-off window. ROSC was considered unachievable if it could not be attained after three attempts of (60 sec) chest compressions after turning the ventilator back on. After 5 min, a second set of 30 sec compressions was administered. This sequence was repeated every 5 min for 60 min to study ROSC, haemodynamic rescue and stabilisation *during each compression phase* every 5 min, for a total time of one hour (see Fig. 4.1). Heart rate, SAP, DAP, and MAP were monitored throughout the study.

Study 2: Effects of AL, lidocaine and saline cold following one set of chest compressions

Rats (300-400 g) were randomly assigned to one of three groups: 1) saline cold, 2) lidocaine cold, and 3) AL cold. Each group consisted of eight animals.

Without induced hypothermia, ROSC could not be sustained after 5 min of resuscitation (see result Chapter 3, Table 3.5). As mentioned in Aim 2, pilot studies showed that one set of 60 sec was sufficient to achieve and sustain ROSC in some hypothermic groups. Thus, the protocol for study 2 was *one set* of 60 sec chest compressions following drug administration (Fig. 4.1), to see if ROSC could be generated and sustained without any further compressions in cold groups. Heart rate, arterial pressures (SAP, DAP and MAP) and ECG stability were continuously monitored for 60 min for each treatment group.

Definitions: ROSC was defined as a SAP >20 mmHg. Cardiac arrest was defined as a MAP <10 mmHg (Fig 4.1). Survival was defined as those animals that achieved ROSC. Total duration of ROSC was defined as the total duration of ROSC (sec) following chest compressions. Episodes and duration of arrhythmias were recorded using lead II ECG and identified using the Lambeth convention, as previously outlined in Canyon and Dobson (2004). Ventricular fibrillation (VF) was defined as QRS deflections that could not easily be distinguished and HR that could not be measured, and ventricular tachycardia (VT) was defined as four or more consecutive ventricular premature beats (premature QRS complexes).

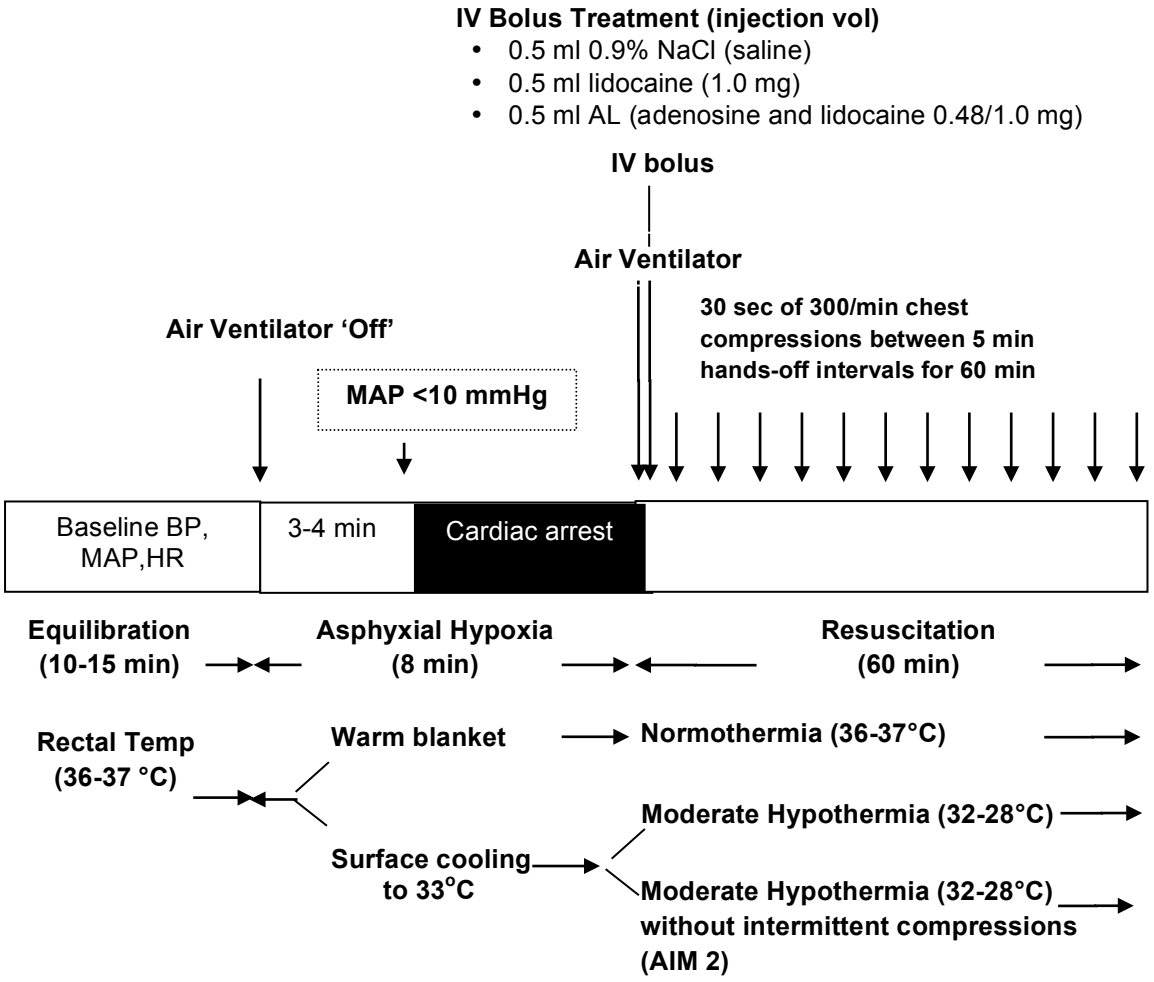


Figure 4.1 A schematic of the experimental protocol with intra-arrest hypothermia application in 8 min asphyxial hypoxia rat model
Cardiac arrest (MAP <10 mmHg) occurred within 3-4 min after turning off the ventilator. Rectal temperature was maintained at 36-37^{o}C throughout the experiments in warm groups, while the cold groups received surface cooling during asphyxial hypoxia to target 33^{o}C during CPR down to 28^{o}C following resuscitation. Intermittent chest compressions with 5 min hands-off interval were applied in warm and cold groups for 60 min. Another series of cold groups only received one (60 sec) chest compression set (Study 2 aim)

4.3.2 Statistical Analysis

Statistical analysis was performed using SPSS statistical package (PASW statistics 18). All values are expressed as mean ± SEM. Each treatment group consisted of eight animals. A post-hoc power analysis was computed with G-Power program (G.Power 3.0.10 software, Germany). Data distribution was tested with *Kolmogorov-Smirnov* for normality. The duration of ROSC was compared using a *Kaplan-Meier* analysis and *Mantel-Cox* log rank. Haemodynamic data (SAP, DAP, MAP, heart rate) were evaluated using a one-way analysis of variance (ANOVA) with *Tukey*'s post-hoc test. Frequency and duration of ventricular arrhythmias were compared using *Kruskal-Wallis*, and followed by pairwise comparisons using a *Mann-Whitney U* test if the analysis was significant. Statistical significance is defined as a $p<0.05$.

4.4 Results

Study 1:

4.4.1 Effects of AL, lidocaine and saline following normothermic and hypothermic intermittent chest compressions

4.4.1.1 Haemodynamic data at baseline and during asphyxia

Baseline haemodynamic data as well as the time and duration of cardiac arrest are presented in Table 4.1. No significance differences were found in haemodynamics during baseline among the control and treatment groups that received intermittent chest compressions (warm and cold groups). SAP during baseline ranged from 104 to 120 mmHg, DAP ranged from 93 to 101 mmHg, MAP ranged from 97 to 109 mmHg, HR ranged from 301 to 337 mmHg, and rectal temperature ranged from 36.2 to 36.6^{o}C.

The time to induce cardiac arrest and its total duration were not significantly different between any groups (Table 4.1). The number of animals that went into asystole during 8 min asphxial hypoxia was almost twofold in normothermic groups (six out of 30; 20%) compared to the hypothermic groups (three out of 27; 11%) (data not shown). These animals were excluded from further analysis and did not receive further treatment.

Table 4.1 Comparison of baseline haemodynamic data, time to cardiac arrest (CA) and the duration of CA for the treatment groups

Group	n	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (bpm)	Rectal temp (°C)	Time to CA (sec)	Duration of CA (sec)
Animals with intermittent compressions								
Sal warm	8	110 ± 3.4	98 ± 4.4	102 ± 4.3	328 ± 6.8	36.6 ± 0.1	225 ± 5.9	255 ± 5.9
Lido warm	8	109 ± 2.7	94 ± 3.0	99 ± 3.0	337 ±10.3	36.3 ± 0.1	232 ± 9.6	247 ± 9.6
AL warm	8	110 ± 6.3	100 ± 6.2	104 ± 6.3	321 ± 5.9	36.5 ± 0.2	227 ± 8.4	252 ± 8.4
Sal cold	8	104 ± 2.3	93 ± 3.8	97 ± 3.2	302 ± 7.6	36.6 ± 0.1	244 ± 7.7	236 ± 7.7
Lido cold	8	120 ± 6.3	94 ± 6.2	109 ± 6.2	320 ± 8.7	36.2 ± 0.2	248 ± 8.8	232 ± 8.8
AL cold	8	112 ± 3.6	101 ± 3.0	105 ± 3.1	301 ±10.6	36.6 ± 0.2	256 ± 9.0	223 ± 9.0

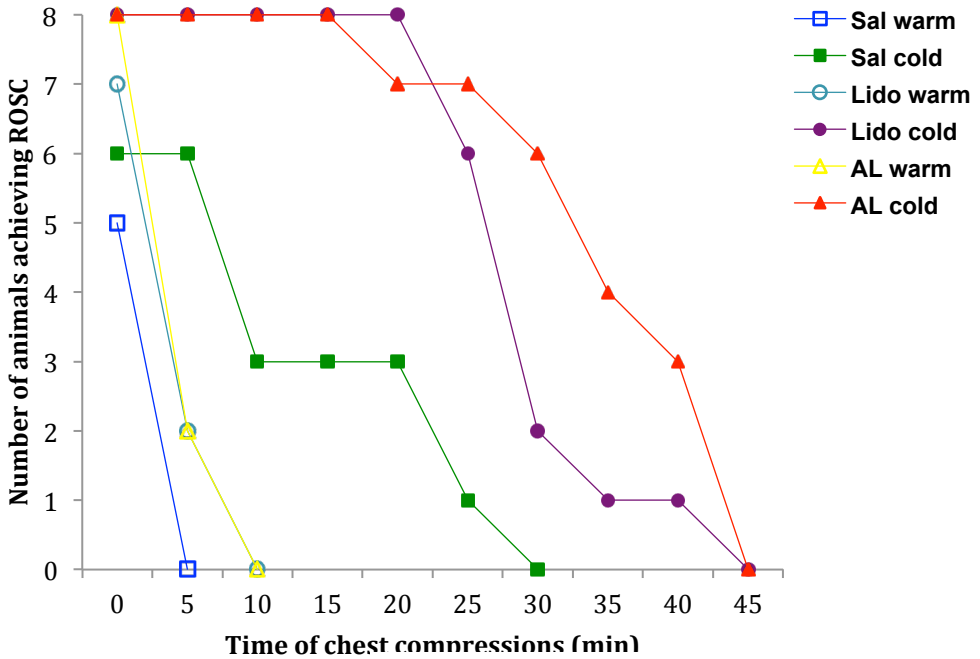
Values represent mean ± SEM. SAP = systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, HR = heart rate, Time to CA = time to cardiac arrest from the initiation of asphyxia to the onset of cardiac arrest (MAP <10 mmHg), Duration of CA =duration of cardiac arrest. No significant difference found among groups.

4.4.1.2 ROSC achievements and total duration of ROSC following intermittent chest compressions

Five (62.5%) of Sal warm, seven (87.5%) of Lido warm and eight (100%) of AL warm animals achieved ROSC following drug injections and the first set of chest compressions (Fig. 4.2A), compared to six (75%) Sal cold and eight (100%) of both Lido and AL cold groups. After five minutes (i.e. after the second set of chest compressions), no (0%) Sal warm and six (75%) Sal cold groups achieved ROSC. ROSC was achieved during each of the 5 min hands-off periods in cold saline controls until 25 min (n=1), while Lido and AL cold groups achieved ROSC up to 40 min. After 30 min, only two Lido cold compared to six AL cold rats achieved ROSC, and one Lido compared to three AL cold rats after 40 min (three times survival window).

The total duration of ROSC for each treatment group is illustrated in Figure 4.2B. None of the warm groups had a total ROSC duration of more than 10 min. In cold groups, the longest total duration of ROSC achieved by Sal group was less than 10 min, while both Lido and AL cold had durations exceeding 35 min. However, the percentage of rats with ROSC duration of more than 20 min was four times higher in AL compared to Lido cold group (50% vs 12.5%) (Fig. 4.2B).

A.



B.

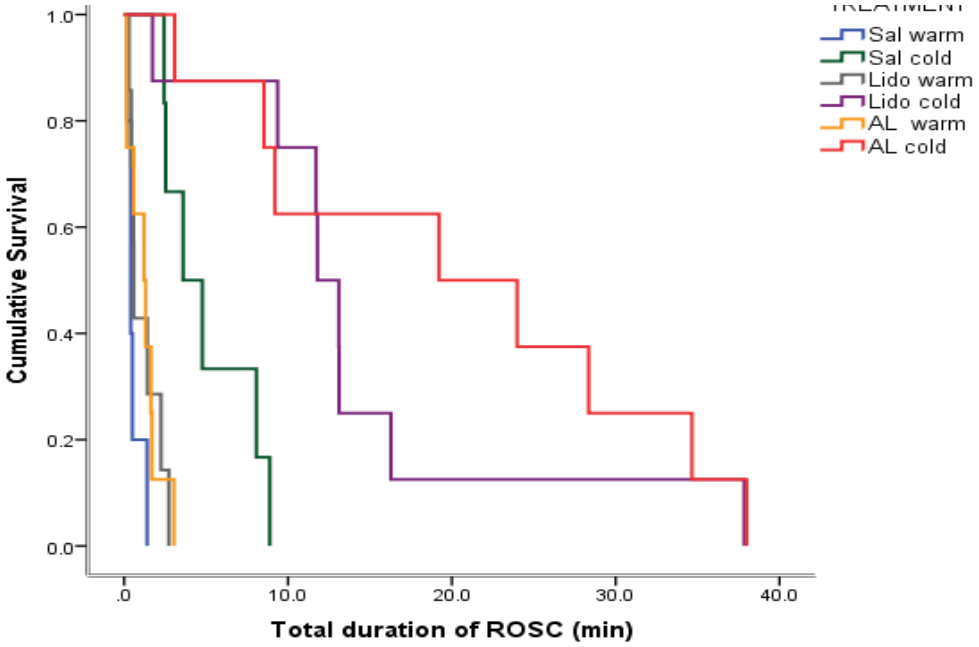


Figure 4.2 ROSC achievements and duration among treatment groups
A. The number of animals achieving ROSC following 30 sec chest compression with 5 min intervals among groups. B. *Kaplan-Meier survival* curves comparing total duration of *ROSC per animal among treatments* (X^2*=56.058 df (5) p<0.01).*

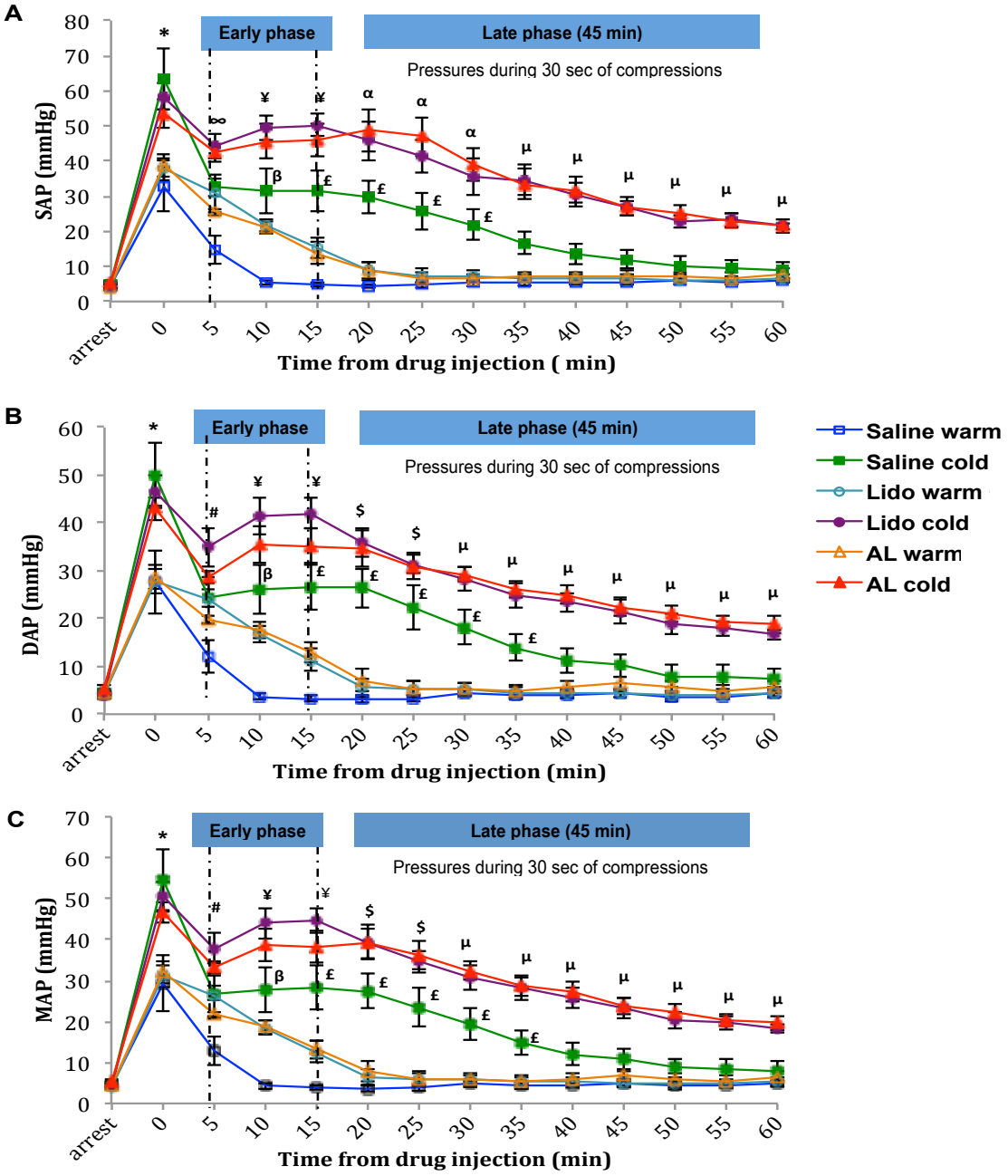


Figure 4.3 Arterial blood pressures during 30 sec of compressions separated by 5 min hands-off periods over 60 min

A. Systolic arterial pressures (SAP), B. Diastolic arterial pressures (DAP), and C. Mean arterial pressures (MAP). Values represent the mean ± SEM. *p<0.05 for Saline cold compared to all warm groups. $^{\infty}$p<0.05 for Lido and AL cold compared to Saline and AL warm. $^{\#}$p<0.05 for Lido cold compared to Saline and AL warm groups; and AL cold compared to saline warm group. $^{¥}$p<0.05 for Lido cold compared to saline cold and all warm groups; and AL cold compared to all warm groups. $^{\beta}$p<0.05 for Saline cold compared to Saline warm. $^{£}$p<0.05 for Saline cold compared to all warm groups. $^{\$}$p<0.01 for Lido and AL cold compared to all warm groups. $^{\mu}$p<0.01 for Lido and AL cold compared to all other groups

4.4.1.3 Haemodynamics during intermittent chest compressions

During the first chest compression set (time 0), arterial pressures (SAP, DAP, MAP) were higher in cold groups, but at this stage, only Sal cold achieved significantly higher pressures compared to all warm groups (Fig. 4.3). Sal warm failed to generate arterial pressures (MAP<10 mmHg) as early as 10 min from the onset of resuscitation, while at the same time, Lido and AL warm groups produced MAPs of 18 ± 1.7 and 19 ± 1.7 mmHg, respectively (Fig. 4.3C). However, arterial pressures in these warm groups fell below 10 mmHg after 20 min.

In cold groups, saline treatment initially produced slightly higher arterial pressures than lidocaine and AL (time 0). However, starting at 5 min, SAP, MAP and DAP produced by cold controls decreased markedly, but these pressures were still significantly improved compared to all warm groups up to 35 min (Fig. 4.3A,B,C). MAP continuously dropped in cold controls during the late phase and fell below 10 mmHg after 50 min. It is noteworthy that Lido and AL cold groups maintained MAP >35 mmHg during the early phase and >18 mmHg up to 60 min. At later phase (30-60 min), arterial pressures were significantly higher in Lido and AL cold rats than any other group including cold controls. At 60 min, SAP and DAP for Lido cold were 22 ± 1.0 and 17 ± 1.0 mmHg, and for AL were 22 ± 2.0 and 19 ± 1.8 mmHg, while all other groups failed to generate SAP and DAP >10 mmHg regardless of chest compressions given (Fig. 4.3A,B).

4.4.1.4 Incidence of arrhythmias during and after intermittent chest compression

The frequency and duration of VT and VF experienced by animals are reported in Figure 4.4 and Table 4.2. Five (63%) and four (50%) out of eight animals in warm and cold controls, respectively, experienced ventricular fibrillation (VF) (Table 4.2), and some of these occurred during the first chest compressions resulting in no ROSC. Warm and cold controls also experienced a large number of ventricular tachycardia (VT), an average of 12 ± 4.5 and 10 ± 4.8 incidences per animal, respectively (Table 4.2). Unlike VF, most VT was non-sustainable but did progress to VF in some cases. There were no significant differences found between control cold and warm groups in VT or VF frequency and duration.

In lidocaine-treated rats, more frequent and longer durations of VT were found in cold compared to warm groups. VT incidences were 4 ± 0.9 and 9 ± 2.3 and the durations

were 10 ± 3.2 and 34 ± 2.9 sec per animal, respectively, in lidocaine warm and cold groups (Fig. 4.4, Table 4.2). These differences were not found to be statistically significant. VF events were also found in two and one animals of lidocaine cold and warm groups, respectively, but the durations were shorter compared to controls. In contrast, although non-sustainable VT (duration up to 10 ± 6.5 sec) occurred, both AL groups showed no VF during chest compressions and hands-off periods over the 60 min monitoring period.

Table 4.2 Comparison of frequency and duration of ventricular arrhythmias among treatment groups

Group	N			Frequency of arrhythmias per animal			Duration of arrhythmias per animal (sec)		
	VT	VF	VT /VF	VT	VF	VT/VF	VT	VF	VT/VF
With intermittent chest compressions									
Sal warm	5	5	6	12 ± 4.5	2 ± 0.2	11 ± 4.0	45 ± 18.5	98 ± 21.4	120 ± 29.5
Sal cold	5	4	6	10 ± 4.8	4 ± 1.0	10± 4.9	36 ± 18.8	125 ± 68.6	113 ± 46.0
Lido warm	3	2	3	4 ± 0.9	1 ± 0.0	4 ± 0.9	10 ± 3.2	33 ± 12.5	31 ± 15.8
Lido cold	6	1	6	9 ± 2.3	1	9 ± 2.3	34 ± 2.9	5	35 ± 13.6
AL warm	3	0	3	4 ± 2.7	0	4 ± 2.7	10 ± 6.5	-	10 ± 6.5*
AL cold	3	0	3	4 ± 2.7	0	4 ± 2.7	6 ± 3.9	-	6 ± 3.9*

Values represent mean ± SEM. Animal without arrhythmias were excluded from analysis. N =number of animals with arrhythmic episodes, VT = ventricular tachycardia, VF = ventricular fibrillation. *p<0.05 compared to Sal warm.

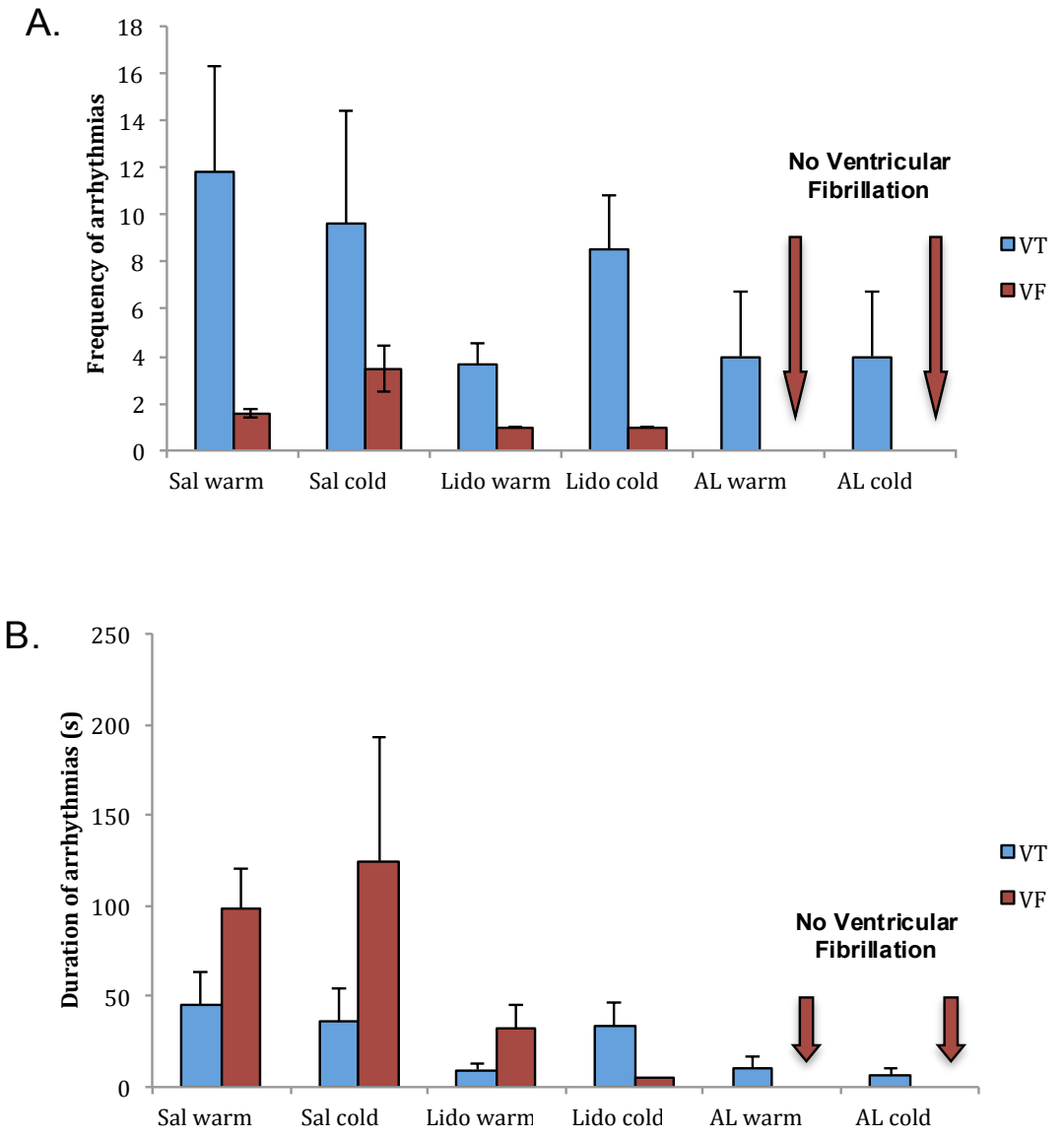


Figure 4.4 Ventricular arrhythmias per animal during hands-off periods
A. Frequency of arrhythmias and B. Duration of arrhythmias. Values represent mean ± SEM.

Study 2:

4.4.2 Effects of AL, lidocaine and saline cold following one set of chest compressions

4.4.2.1 Haemodynamic data at baseline and during asphyxia

Baseline haemodynamic and cardiac arrest data are presented in Table 4.3. No significance differences were found in haemodynamics during baseline among the control and treatment groups. SAP during baseline ranged from 123 to 129 mmHg, DAP ranged from 107 to 112 mmHg, MAP ranged from 112 to 117 mmHg, HR ranged from 310 to 339 bpm, and rectal temperature ranged from 36.0 to 36.1^{o}C.
The time to induce cardiac arrest ranged from 217 to 231 sec and its total duration ranged from 253 to 257 sec, and there were no significant differences between groups (Table 4.3).

Table 4.3 Comparison of baseline haemodynamic data, time to cardiac arrest (CA) and duration of CA for the treatment groups

Group	n	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	Heart rate (bpm)	Rectal temp (°C)	Time to CA (sec)	Duration of CA (sec)
Without intermittent compressions								
Sal cold	8	124 ± 3.5	110 ± 3.1	114 ± 3.1	339 ± 9.5	36.1 ± 0.1	231 ± 9.1	254 ± 7.0
Lido cold	8	129 ± 2.7	112 ± 2.2	117 ± 2.3	330 ± 8.0	36.0 ± 0.1	220±12.5	257 ± 5.0
AL cold	8	123 ± 3.7	107 ± 4.4	112 ± 4.0	310 ±10.9	36.0 ± 0.2	217 ± 7.8	253 ± 7.8

Values represent mean ± SEM. SAP = systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, HR = heart rate, Time to CA = time to cardiac arrest from the initiation of asphyxia to the onset of cardiac arrest (MAP <10 mmHg), Duration of CA =duration of cardiac arrest. No significant difference found among groups.

4.4.2.2 ROSC achievement and duration in cold groups without intermittent chest compressions

Eight (100%) of AL cold, seven (87.5%) of Lido cold, and six (75%) of Sal cold achieved ROSC after one set of chest compressions, and, once achieved, this was sustained until the end of the experimental period (60 min) (Fig. 4.5).

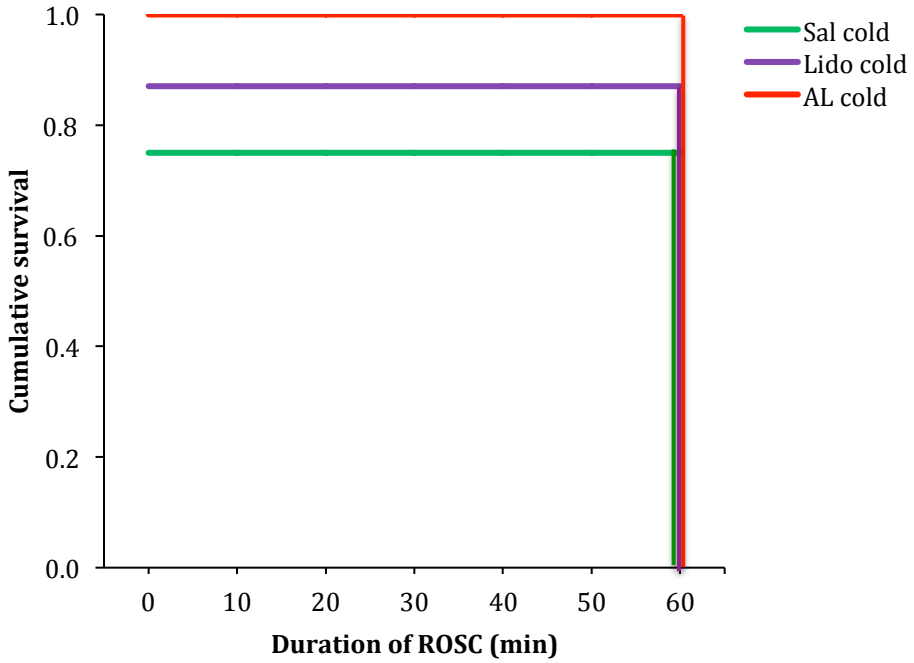


Figure 4.5 Total duration of ROSC per animal among treatments with one-set of chest compressions

4.4.2.3 Haemodynamics in cold groups without intermittent chest compressions

The haemodynamics during ROSC in hypothermia groups are presented in Figure 4.6. Immediately following resuscitation, SAP of Sal and AL cold were similar (79 ±17.0 vs 71 ± 6.7 mmHg), however, DAP of AL was lower than Sal cold (50 ± 14.6 vs 22 ± 2.5), resulting in a significantly higher pulse pressure in AL cold (49 ± 6.6 mmHg) compared to Sal cold (29 ± 2.8 mmHg) or Lido cold (27 ± 2.6 mmHg) and this occurred at a significantly low heart rate (<50% of Sal and Lido cold groups) (Fig.4. 6). The MAP of Sal cold rapidly increased after 15 min (from 54 ± 6.3 at 10 min to 107 ± 10.7 mmHg at 20 min) and was significantly higher than the other groups at this time. In contrast, MAP of Lido and AL cold groups peaked after 30 min at 84 ± 4.4 and 79 ± 7.0 mmHg, respectively. From 30-60 min, there were no significant differences in MAPs between all cold groups, but the heart rate of AL cold was markedly lower than saline cold (statistically significant at 45 and 55 min) (see Fig. 4.6 D).

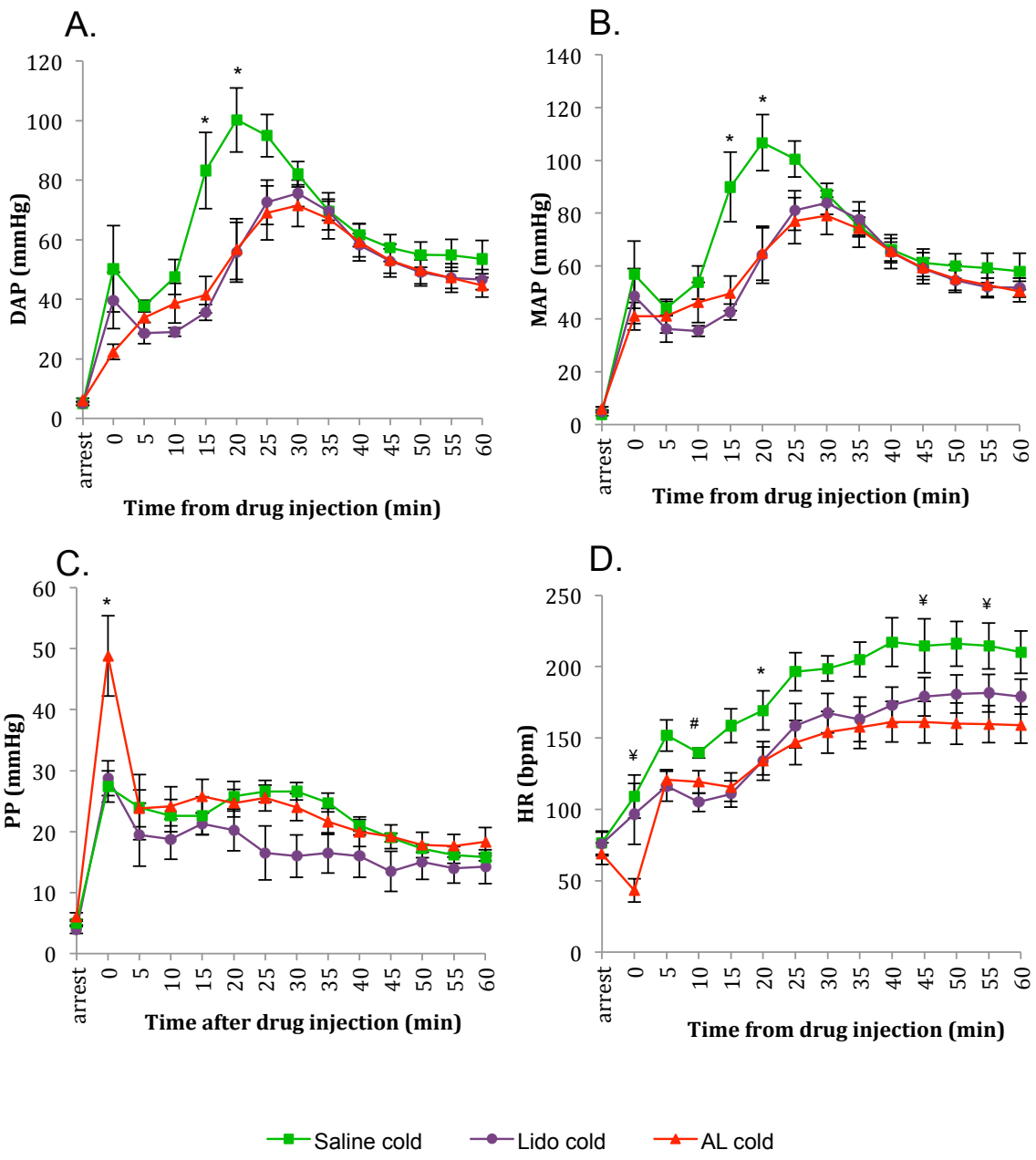


Figure 4.6 Haemodynamics in cold groups following drug injection without intermittent chest compressions over 60 min

A. Diastolic arterial pressures (DAP), B. Mean arterial pressures (MAP), C. Pulse pressures (PP), and D. Heart rate (HR). Values represent the mean ± SEM.

* p<0.05 for Saline cold compared to all other groups

# p<0.05 for Saline cold compared to Lido cold only

¥ p<0.05 for Saline cold compared to AL cold only

4.4.2.4 Incidence of arrhythmias in cold groups without intermittent chest compressions

With one continuous set of chest compressions lasting 60 sec, only saline cold rats (four out of eight) experienced VF which lasted 142 ± 55 sec (Table 4.4), while AL and Lido cold groups demonstrated no VF at any time. Two rats in the saline cold group had a VT incidence of three events, while non-sustainable VT was experienced only once in two lidocaine cold rats and one AL cold (Table 4.4). The duration of the single VT experienced in AL was considerably shorter (1 sec) compared to 25 ± 7.0 sec in the lidocaine cold group.

Table 4.4 Comparison of frequency and duration of ventricular arrhythmias among treatment groups

Group	N			Frequency of arrhythmias per animal			Duration of arrhythmias per animal (s)		
	VT	VF	VT/ VF	VT	VF	VT/VF	VT	VF	VT/VF
Without intermittent chest compressions									
Sal cold	2	4	4	3 ± 1.0	2 ± 0.6	4 ± 1.5	10 ± 6.0	142 ± 55	149 ± 56.4
Lido cold	2	0	2	1 ± 0.5	0	1 ± 0.5	25 ± 7.0	-	25 ± 7.0
AL cold	1	0	1	1	0	1	1 ± 0	-	1

Values represent mean ± SEM. N = number of animals with arrhythmic episodes, VT = ventricular tachycardia, VF = ventricular fibrillation, VT/VF = ventricular tachycardia and/or fibrillation.

4.5 Discussion

The data from Study 1 presented in the first part of this Chapter shows that no treatment or control group could achieve sustainable ROSC after 5 to 10 min at normothermic temperatures (36-37°C), and confirms what was reported in Chapter 3 for accidental hypothermic rats (34-35°C). In contrast, body cooling led to ROSC achievement in all groups but treatments differed in their response to chest compressions. No saline cold controls achieved ROSC after 30 min of chest compressions, whereas two lidocaine- and six AL-treated cold rats achieved ROSC. During these 30 min, AL and Lido groups generated similar MAPs and both were significantly higher than saline controls (Fig. 4.3). AL rats showed no VF, Lido had an average of one VF and controls had four VF episodes. In the second part of the Chapter, it was shown that after one set of 60 sec of compressions, 100% of AL-treated cold animals could generate ROSC and sustain it for 60 min compared to 87.5% Lido-treated rats, and 75% saline controls. During ROSC, AL-treated rats had a ~10% lower heart rate than Lido rats but it was not significant. AL but not Lido-treated rats had significantly lower heart rates than saline controls at ROSC and after 45 min post-ROSC. These findings will now be discussed.

4.5.1 Study 1: The effect of normothermia vs hypothermia during intermittent chest compressions

4.5.1.1 During normothermia, no ROSC was attained in any group after 10 min and AL and lidocaine resuscitations were equivalent during chest compressions

In the present study, no groups at normal body temperature could generate ROSC after 5 to 10 min (Fig. 4.2 A). This finding is consistent with Tsai and colleagues who compared ROSC attainment in normothermic asphyxial and VF cardiac arrest models (Tsai*, et al.*, 2012). In the asxphxial model, they found only one out of ten male Wistar rats achieved ROSC after 60 sec of resuscitation compared to four out of ten animals in the VF group. They further showed that asphyxial cardiac arrest presented more diffuse myocardial injuries and more severe mitochondrial damage than VF cardiac arrest (Tsai*, et al.*, 2012). From the data in this Chapter, it was concluded that neither AL, lidocaine nor saline at warm temperatures improved the incidence of ROSC after 5 to 10 min. As discussed in the previous chapter, it would be interesting to know what the coronary perfusion pressure (CPP) was in these groups, as patients with CPP <15 mmHg generally do *not achieve* ROSC *(Paradis, et al., 1990)*.

Despite the inability of any group to achieve ROSC after 5-10 min, chest compressions were continued every 5 min for 60 min. It was found that, SAP, DAP and MAP during chest compressions were consistently higher in the warm AL and lidocaine treated-rats compared with saline over the 60 min period. In contrast to Chapter 3, AL was not consistently higher than lidocaine or saline groups. This difference may be due to a difference in the maintenance of body temperature between the two studies. A lower accidental core temperature of 1°C in Chapter 3 may be important for AL treatment's effectiveness, considering 1^{o}C drop in body temperature may affect cardiac and neurological function (Bernard*, et al.*, 1997; Ehrlich*, et al.*, 2002). From the data in the present chapter it was concluded that no animal could sustain ROSC after 10 min during normothermia, and that AL and lidocaine treatments were equivalent and developed higher arterial pressures than saline controls in the first 15 min, but the differences were not significant ($p<0.05$). The clinical significance of accidental versus active maintenance of normothermia will be addressed in the general discussion (Chapter 8, section 8.3.1).

4.5.1.2 Mild hypothermia improved AL ROSC outcomes with equivalent haemodynamics to lidocaine treatment during compressions

In direct contrast to the warm groups, mild hypothermia significantly improved ROSC in all animals as well as haemodynamic function. Although hypothermia is important to improve the chance of ROSC, different responses were observed with drug treatments during cold and AL led to a significantly higher survival chance compared to saline or lidocaine after prolonged chest compressions. No saline cold controls achieved ROSC after 30 min of chest compressions, while two lidocaine-treated rats and six AL-treated cold rats achieved ROSC. It is shown from *Kaplan-Meier* analysis that AL was significantly superior in attaining ROSC at mild to moderate hypothermia *(X^2=56.058 df (5) p<0.01)* (see Fig. 4.2).

The higher incidence of ROSC in all cold groups was related to higher arterial pressures generated during chest compressions. A higher DAP was particularly noteworthy as it has been experimentally associated with improved coronary blood flow and ROSC achievement (Yannopoulos*, et al.*, 2013). For example, in the absence of any therapeutic treatment (AL or lidocaine), the DAP in saline controls doubled from 12 ± 3 mmHg to 24 ± 2 mmHg after the second set of chest compressions (t=5 min) during normothermic versus hypothermic resuscitation, respectively (Fig 4.3B). It was

found that during chest compressions, a minimum DAP of 20 mmHg was required to stimulate ROSC during release in any group under normothermia or hypothermia (data not shown). Further experiments are required to establish the relationship between developed pressures, in particular DAP, and coronary perfusion pressure (CPP) with successful ROSC (Duchateau*, et al.*, 2010).

Improvements of ROSC achievement and haemodynamics during mild-to-moderate hypothermia in all three groups may be related to: 1) an energy-demand lowering effect (Bernard & Buist, 2003), 2) improved cardiac contractility (Groban*, et al.*, 2002), believed due to improved sarcoplasmic reticulum and mitochondrial Ca^{2+} handing (Stowe*, et al.*, 1999), 3) increased peripheral vascular resistance (Bernard*, et al.*, 2002; Filseth*, et al.*, 2010), and 4) greater electrical stabilisation during arrest prior to resuscitation from slowing myocardial and ECG deterioration as found in the swine model of VF-induced cardiac arrest (Menegazzi*, et al.*, 2009).

4.5.1.3 AL treatment led to no VF during hypothermia or normothermia

A recurring result in Chapter 3 and 4 is that, in contrast to all other groups, AL led to no VF, regardless of the temperature condition. Although lidocaine treatment reduced the incidence compared to saline controls, one animal developed VF during both warm and cold resuscitation. Previously in Chapter 3, AL treatment showed superior ECG rhythm stabilisation during chest compressions (no VF, and reduced VT events) compared to adenosine, lidocaine and saline treatment. Five (62.5%) and four (50%) out of eight saline controls in warm and cold groups, respectively, experienced two to four episodes of VF per animal.

The possible mechanisms for the anti-arrhyhthmic properties of AL were discussed in Chapter 3 (section 3.5.2), and possible clinical use of AL or lidocaine are discussed in the second part of this discussion.

4.5.2 Study 2: The effect of hypothermia with one set of 60 sec chest compressions

4.5.2.1 ROSC attainment and post-ROSC haemodynamics in AL, lidocaine and saline controls with one set of chest compressions

After a single set of 60 sec chest compressions, six of eight (75%) saline controls, seven of eight (87.5%) lidocaine-treated, and eight of eight (100%) AL-treated rats immediately achieved ROSC and maintained it for 60 min without further compressions. Thus, hypothermia applied from arrest through to post-resuscitation was directly responsible for achievement and sustainability of ROSC in all groups, and while AL had a 100% success rate, the result was not significant from lidocaine or saline controls, probably due to small sample size (n=8). These results may have clinical significance as mild therapeutic hypothermia *before ROSC* has been shown to be feasible in animal and human studies (Scolletta*, et al.*, 2012), and the emerging data suggest improvements in cardiac and neurological functions compared with normothermia and/or conventional hypothermia in experimental models of cardiac arrest (Abella*, et al.*, 2004; Tsai*, et al.*, 2008; Zhao*, et al.*, 2008) and also humans . The present data would support this proposition.

In AL-treated rats, ROSC was initiated at acutely low heart rates and significantly higher pulse pressures than in the lidocaine group or saline controls (Fig 4.6). A reduced heart rate has been associated with small volume hypertonic saline with AL (with Mg^{2+}) resuscitation therapy in a number of trauma states including small-volume resuscitation in pigs following 75% blood loss and 90 min of shock (Granfeldt*, et al.*, 2014). A lower heart rate during AL hypotensive resuscitation in the pig was associated with a twofold increase in stroke volume at 60 min, and an increase in systolic ejection time. The authors argued that the lower heart rate and increased systolic ejection time increased stroke volume by permitting greater volumes of blood in the LV to be ejected per beat compared to hypertonic saline controls (Granfeldt*, et al.*, 2014). This unique mechanism to increase stroke volume in the pig at lower heart rates may contribute to AL's ability to resuscitate from asphyxial hypoxia as seen in this study but ultrasound echocardiographic measurements or pressure-volume loops would be required at baseline and post-ROSC states to evaluate this hypothesis. Interestingly, in the present chapter, the higher pulse pressure would also reflect a higher stroke volume (Augusto*, et al.*, 2010). A decreased heart rate is also associated with reduced oxygen consumption (Reil & Böhm, 2007) and increased cardiac filling time (Sherwood, 2013).

4.5.2.2 Incidence of arrhythmias with AL, lidocaine and saline treatments with one set of chest compressions

Similar to intermittent chest compressions during hypothermia (Study 1), there was no VF found in AL or lidocaine groups. However, 50% of saline controls experienced VF, with two of them occurring during chest compressions and resulting in no ROSC during release (t=0). In addition, slightly reduced VT incidences following ROSC were also found in AL and Lido cold compared to saline cold group (Table 4.4). However, the VT incidence in the AL–treated rat was much shorter (1 sec) than Lido cold (25 sec; n=2). Again, AL shows a consistent reduction of severe arrhythmias but was not significantly different from lidocaine.

Lidocaine has a checkered history in cardiac resuscitation as outlined in Chapter 1. Briefly, in the 1980s, lidocaine was recommended as the first choice of anti-arrhythmic drugs in resuscitation guidelines (Kudenchuk & Racht, 1999). In the 1990s it went out of favour due to a lack of prospective randomized clinical trial data showing a benefit of lidocaine in cardiac arrest patients (Neumar*, et al.*, 2010). However, randomized trials in 2013 showed that lidocaine was more beneficial in younger patients (<18 years old) than amiodarone (Valdes*, et al.*, 2014), while amiodarone use was not associated with superior rates of ROSC or survival at 24h. Nevertheless, neither drug was associated with survival to hospital discharge (Valdes*, et al.*, 2014), and further studies are required (Kudenchuk*, et al.*, 2013). Following on, from the interesting results presented in this chapter it would be interesting to include AL in future trials.

4.5.3 Limitations of the study

In Study 1 presented in the first part of this chapter, intermittent chest compressions were continued in hypothermic rats even though ROSC was achieved after the first set of compressions. This protocol was followed to validly compare the haemodynamics under identical conditions in cold and warm conditions. Of course, in clinical practice, chest compressions would not be performed on a subject that has already attained ROSC.

It was found in Study 1 that 5 min intermittent chest compressions during hypothermia did compromise the haemodynamics in all groups compared to the one-set chest compression protocol of Study 2. This may have occurred from cardiac dysfunction or due to including injuries to the thoracic, abdominal visceral and/or pulmonary area (Hashimoto*, et al.*, 2007). Although Hashimoto et al. (2007) found the injury incidence

to be quite low (~2%), a more recent study of While and colleagues (White*, et al.*, 2010) reported thoracic complications in 20-60% of patients (Corbett & O'Callaghan, 1997; Krischer*, et al.*, 1987; Sommers, 1991). However, the life-saving benefits of chest compressions appear to outweigh the damage it costs in cardiac arrest patients (Rajab*, et al.*, 2011).

Application of intra-arrest hypothermia in this study may be logistically challenging in pre-hospital setting, but it may find clinical importance for in-hospital arrest since intra-arrest cooling appears to be feasible, effective, and safe in animals and humans (Scolletta*, et al.*, 2012). Another limitation is the short monitoring period of 60 minutes. Further studies are required with longer observation periods to examine whether the haemodynamic stability during ROSC could be maintained with hypothermia, AL and lidocaine past 60 minutes.

4.6 Conclusion

This chapter showed that no treatment achieved ROSC after 5 to 10 min at normothermic temperatures (36-37°C). In direct contrast to the warm groups, mild hypothermia significantly improved ROSC and haemodynamic function in all animals. A *Kaplan-Meier* analysis showed that AL was significantly superior in attaining ROSC at mild to moderate hypothermia. AL also provided improved protection from arrhythmic events, especially VF, during intermittent chest compressions (warm or cold). In the second part of the chapter, after a single set of 60 sec chest compressions, six of eight (75%) saline controls, seven of eight (87.5%) lidocaine-treated and eight of eight (100%) AL-treated rats immediately achieved ROSC and maintained it for 60 min without further compressions. Further experiments are required to establish the clinical relevance of AL therapy with hypothermia for cardiac arrest resuscitation.

CHAPTER 5. EFFECT OF MAGNESIUM WITH ADENOSINE, LIDOCAINE AND AL ON ROSC, HAEMODYNAMICS, ECG STABILITY AND COAGULATION STATUS DURING MODERATE HYPOTHERMIA

5.1 Introduction

In Chapter 4 it was shown that mild-to-moderate hypothermia was a powerful protection strategy in the rat model of 8-min asphyxial hypoxia. In addition, small volume AL and lidocaine conferred further resuscitation protection compared to saline controls over a 60 min period. AL and lidocaine showed significantly improved incidence of ROSC and developed haemodynamics during intermittent chest compressions using mild-to-moderate hypothermia compared to normothermia. During hypothermia, AL-treatment led to 100% of ROSC initiated at significantly lower heart rates than all other groups.

In the present chapter, the effect of adding magnesium sulphate ($MgSO_4$) to adenosine, lidocaine and AL solutions will be examined with the goal to improve ROSC and post-ROSC haemodynamics during moderate hypothermia (28-32^{o}C). Magnesium is of interest because recently Letson and colleagues showed that Mg^{2+} added to AL solution (ALM) significantly improved post-resuscitation haemodynamics and survival following haemorrhagic shock in the rat (Letson & Dobson, 2011a). Most surprisingly, they showed that ALM, but not AL alone, fully corrected acute traumatic coagulopathy in this model (Letson*, et al.*, 2012). Therefore, it is hypothesised that ALM may also improve the incidence of ROSC and post-ROSC haemodynamics and correct coagulopathy as assessed by prothrombin time (PT) and *activated partial thromboplastin time* (aPTT) during moderate hypothermia following asphyxial-induced cardiac arrest in the rat.

5.2 Aim

This chapter aimed to examine the effects of Mg^{2+} addition to adenosine (AM), lidocaine (LM) and AL (ALM) solutions, as well as AL and Mg^{2+} alone on ROSC achievements, post-ROSC haemodynamics, ECG stability, and coagulation status during continued induction of hypothermia (28-32^{o}C) following 8 min asphyxial hypoxia in non-heparinised rats.

5.3 Experimental Protocols

5.3.1 Experimental design

Animal housing, feeding and water regimes, ethics approvals, methods of anaesthesia and surgical protocols are described in Chapter 2. Rats (350-400g, n=48) were randomly assigned to one of six groups: 1) saline (SAL), 2) Mg^{2+} (Mg), 3) adenosine-Mg^{2+} (AM), 4) lidocaine-Mg^{2+} (LM), 5) adenosine-lidocaine (AL), and 6) adenosine-lidocaine-Mg^{2+} (ALM) (see Fig. 5.1). The doses used for this study are shown in Fig 5.1 and the concentration of $MgSO_4$ was based on the studies of Letson and colleagues (Letson & Dobson, 2011a).

Rats were anaesthetised, surgically prepared and stabilised for 10-15 minutes before assessing the baseline haemodynamic data as described in Chapter 2. Cardiac arrest was induced by turning off and clamping the tracheotomy tube from the ventilator for eight minutes. Surface cooling (ice-cold pack and ethanol spray) was applied during asphyxiation to target 33^{o}C at the time of resuscitation and continued to drop to 28^{o}C by the end of observation period. Moderate hypothermia was chosen since it was found to improve ROSC achievement and sustainability after asphyxial cardiac arrest in the rat (Chapter 4). In addition, moderate hypothermia (<32^{o}C) is reported to occur in 27% to 63% cardiac arrest patients subjected to surface cooling (Gillies*, et al.*, 2010; Merchant*, et al.*, 2006). Temperature this low has a number of clinically adverse effects, such as depressed cardiac function, decreased haemodynamics and increased risk of coagulopathy (Moore*, et al.*, 2011; Nielsen*, et al.*, 2009).

Resuscitation was commenced by declamping the ventilation tube and injecting 0.5 ml bolus of drugs IV over a 10 second period before restarting the ventilator (at the stroke rate of 95-100/min) followed by chest compressions (300/min) for 60 sec. If ROSC was not achieved, another 30 sec of compressions were performed, followed by a third and last attempt if that effort failed to produce ROSC. No vasopressors were applied during or post-resuscitation. Heart rate and arterial pressures (SAP, DAP and MAP) and ECG stability under moderate hypothermia were continuously monitored for 120 min for each treatment group. After 120 min post-ROSC hypothermia, blood samples were withdrawn and coagulation status was measured using PT and aPTT analysis as described in Chapter 2. In addition to the six treatment groups, blood samples were also obtained from eight healthy rats that underwent moderate hypothermia for two hours but no asphyxia (sham).

Definitions: ROSC was defined as a SAP>20 mmHg. Cardiac arrest was defined as a MAP <10 mmHg (Fig. 5.1). Survival was defined as those animals that achieved and maintained ROSC up to two hours. Episodes and duration of arrhythmias were recorded using lead II ECG and identified using the Lambeth convention, as previously outlined in Canyon and Dobson (2004) and the study detailed in Chapter 3. Ventricular fibrillation (VF) was defined as QRS deflections that could be not easily be distinguished and HR that could not be measured, and ventricular tachycardia (VT) was defined as four or more consecutive ventricular premature beats (premature QRS complexes). Meanwhile, pulseless electrical activity (PEA) was defined as the presence of organised electrocardiographic activity without palpable arterial pressures, and asystole as the absence of any ventricular electrical activity (Kudenchuk*, et al.*, 2012).

5.3.2 Statistical Analysis

Statistical analysis was performed using SPSS statistical package (PASW statistic 18). All values are expressed as mean ± SEM. Data distribution was tested with *Kolmogorov-Smirnov* for normality. Haemodynamic data (SAP, DAP, MAP, heart rate) were analysed for homogeneity of variances followed by one-way ANOVA. Significance was determined with *Tukey's/Dunnett's* post-hoc test. Survival was estimated with *Chi-Square* analysis. Frequency and duration of ventricular arrhythmias were compared using *Kruskal-Wallis.* Statistical significance is defined as a P value of <0.05.

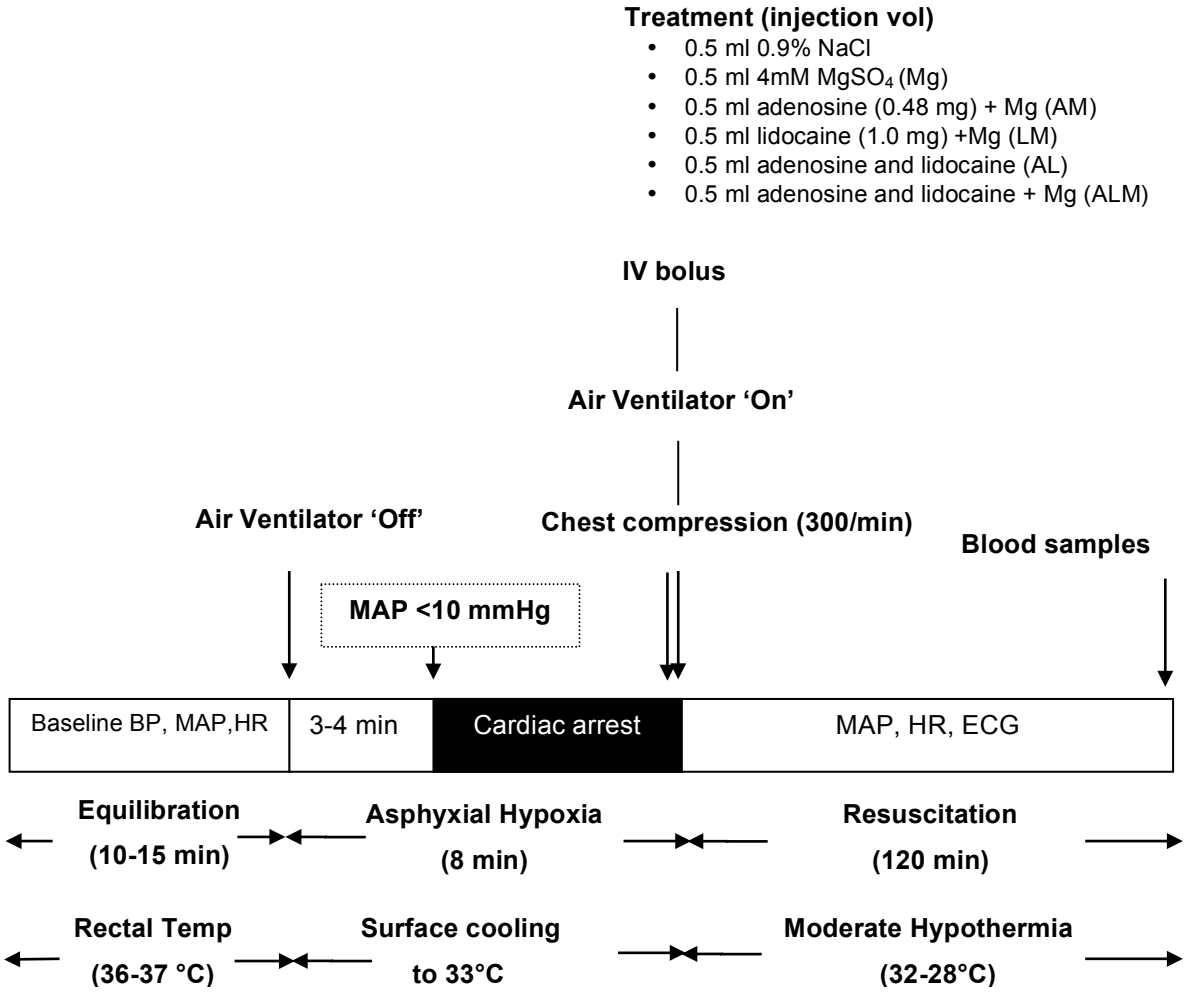


Figure 5.1 A schematic of the experimental protocol application in 8 min asphyxial hypoxia rat model
The animals received surface cooling during asphyxial hypoxia to target 33^{o}C at the onset of resuscitation, and a reduction to 28^{o}C post-resuscitation. Resuscitation attempt comprises IV bolus of drug, declamping ventilator tube, and 60 sec chest compressions. Post-ROSC haemodynamics and ECG rhythm were recorded until 120 min post resuscitation. Blood samples were withdrawn at the end of experiment (t=120 min). An additional n=8 animals had blood withdrawn following 120 min moderate hypothermia with no other intervention for coagulation analysis (sham).

5.4 Results

5.4.1 Baseline and cardiac arrest data among treatment groups

No significance differences were found in baseline haemodynamic data between treatment groups, as well as time to induce and total duration of cardiac arrest (Table 5.1). SAP during baseline ranged from 126 to 138 mmHg, DAP ranged from 112 to 123 mmHg, MAP ranged from 116 to 128 mmHg, and HR ranged from 362 to 386 bpm, and rectal temperature ranged from 36.0 to 36.1^{o}C. Time to cardiac arrest ranged from 206 to 244 sec and cardiac arrest duration ranged from 251 to 265 sec.

Table 5.1 Baseline haemodynamics, rectal temperature, time to and duration of cardiac arrest (CA) among treatment groups

Group	n	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (bpm)	Temp (^{o}C)	Time to CA (sec)	Duration of CA (sec)
SAL	8	130 ± 5.2	118 ± 4.6	122 ± 4.8	386 ± 7.3	36.1 ± 0.1	242 ±12.4	251 ± 5.6
Mg	8	126 ± 2.8	112 ± 2.1	116 ± 2.0	378 ± 7.5	36.0 ± 0.1	218 ± 7.9	265 ± 5.9
AM	8	133 ± 3.0	120 ± 3.1	124 ± 3.0	367 ± 6.2	36.0 ± 0.1	231 ± 12.2	258 ± 6.5
LM	8	138 ± 1.8	123 ± 2.1	128 ± 2.0	383 ± 6.1	36.0 ± 0.0	206 ± 15.9	265 ± 7.1
AL	8	134 ± 2.9	119 ± 3.7	124 ± 3.3	369 ± 9.9	36.0 ± 0.1	208 ± 8.3	264 ± 7.0
ALM	8	138 ± 3.4	121 ± 4.2	126 ± 3.9	362 ± 8.4	36.0 ± 0.1	244 ± 13.3	255 ± 6.3

Values are expressed as mean ± SEM. SAP =systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, HR = heart rate, Temp = rectal temperature, Time to CA = time to cardiac arrest from the initiation of asphyxia to the onset of cardiac arrest (MAP <10 mmHg), Duration of CA = time from cardiac arrest to resuscitation attempt. No significant difference found between groups.

5.4.2 ROSC achievements following resuscitation with different drug treatments

ROSC was achieved in all AL- and ALM-treated animals, while two animals in SAL (25%), three in Mg (37.5%), four in AM (50%), and one in LM (12.5%) groups did not attain ROSC following chest compressions (Table 5.2). The ECG rhythm immediately after chest compressions in non-ROSC animals was different depending on the treatment. While saline-treated animals experienced VF, animals injected with Mg, AM, and LM presented with PEA or asystole. Among animals that achieved ROSC, only one animal (from the AM group) did not survive within the two-hour observation period, and was not included in the post-ROSC haemodynamic analysis.

Table 5.2 The effects of different treatments on resuscitation outcomes following 8 min asphyxial-induced cardiac arrest

Group	Survival		Mortality	
	ROSC, n(%)	2hr, n(%)	No ROSC, n(%)	Cardiac rhythm following treatment
SAL	6 (75)	6 (75)	2 (25)	VF
Mg	5 (62.5)	5 (62.5)	3 (37.5)	PEA/Asystole
AM	4 (50)	3 (37.5)	4 (50)	PEA/Asystole
LM	7 (87.5)	7 (87.5)	1 (12.5)	Asystole
AL	8 (100)	8 (100)	-	-
ALM	8 (100)	8 (100)	-	-

Values are expressed as mean ± SEM. ROSC = return of spontaneous circulation, VF = ventricular fibrillation, PEA =pulseless electrical activity.

5.4.3 Post ROSC haemodynamics during moderate hypothermia among treatment groups

The arterial pressures at ROSC are shown in Table 5.3, and post-ROSC SAPs, DAPs, and MAPs over 120 min are illustrated in figure 5.2, A to C. Immediately after ROSC, SAPs developed were similar in SAL, Mg, LM, AL and ALM groups (ranging from 68-73 mmHg); while the DAPs of the AL and ALM group were 24 ± 2.0 and 34 ± 5.4 mmHg, respectively, which was lower than that of SAL, Mg and LM groups with DAPs of 43 ± 10.3, 45 ± 4.8, and 46 ± 10.8 mmHg, respectively. This resulted in higher PPs in AL and ALM groups (45 ± 5.6 and 39 ± 4.6 mmHg, respectively) compared to all other groups (≤29 mmHg).

Table 5.3 Haemodynamics immediately at ROSC among treatment groups following 8 min asphyxial-induced cardiac arrest

Group	Blood pressures at ROSC			
	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	PP (mmHg)
SAL	72 ± 12.0	43 ± 10.3	50 ± 9.1	29 ± 2.0
Mg	73 ± 8.8	45 ± 4.8	54 ± 6.0	28 ± 4.5
AM	64 ± 6.5	38 ± 12.5	46 ± 9.8	26 ± 7.1
LM	72 ± 13.2	46 ± 10.8	54 ± 11.7	26 ± 3.0
AL	68 ± 5.3	24 ± 2.0	40 ± 3.9	45 ± 5.6
ALM	72 ± 4.7	34 ± 5.4	46 ± 4.5	39 ± 4.6

Values are expressed as mean ± SEM. SAP = systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure. PP = pulse pressure.

At 15 min, DAP and MAP in SAL group markedly increased leading to significantly higher DAP and MAP compared to the AL group. MAPs of all groups increased steadily and peaked after 30 min. At this stage (t=30 min), MAP in the ALM group increased to 108 ± 4.9 mmHg and was significantly higher compared to all other groups (Fig 5.2C). From this point onwards, the arterial pressures (SAPs, DAPs, MAPs) in ALM-treated rats were consistently higher than any other group, and reached statistical significance compared to all other groups from 90 to 120 min (MAPs ranged from 70-76 mmHg). At this late phase, saline-, LM-, AM- and AL-treated rats had MAPs ranging from 47- 59 mmHg. In contrast, Mg-treated rats had the lowest arterial pressures, with MAPs ranging from 41-46 mmHg 90-120 min following ROSC (see Fig. 5.2A-C).

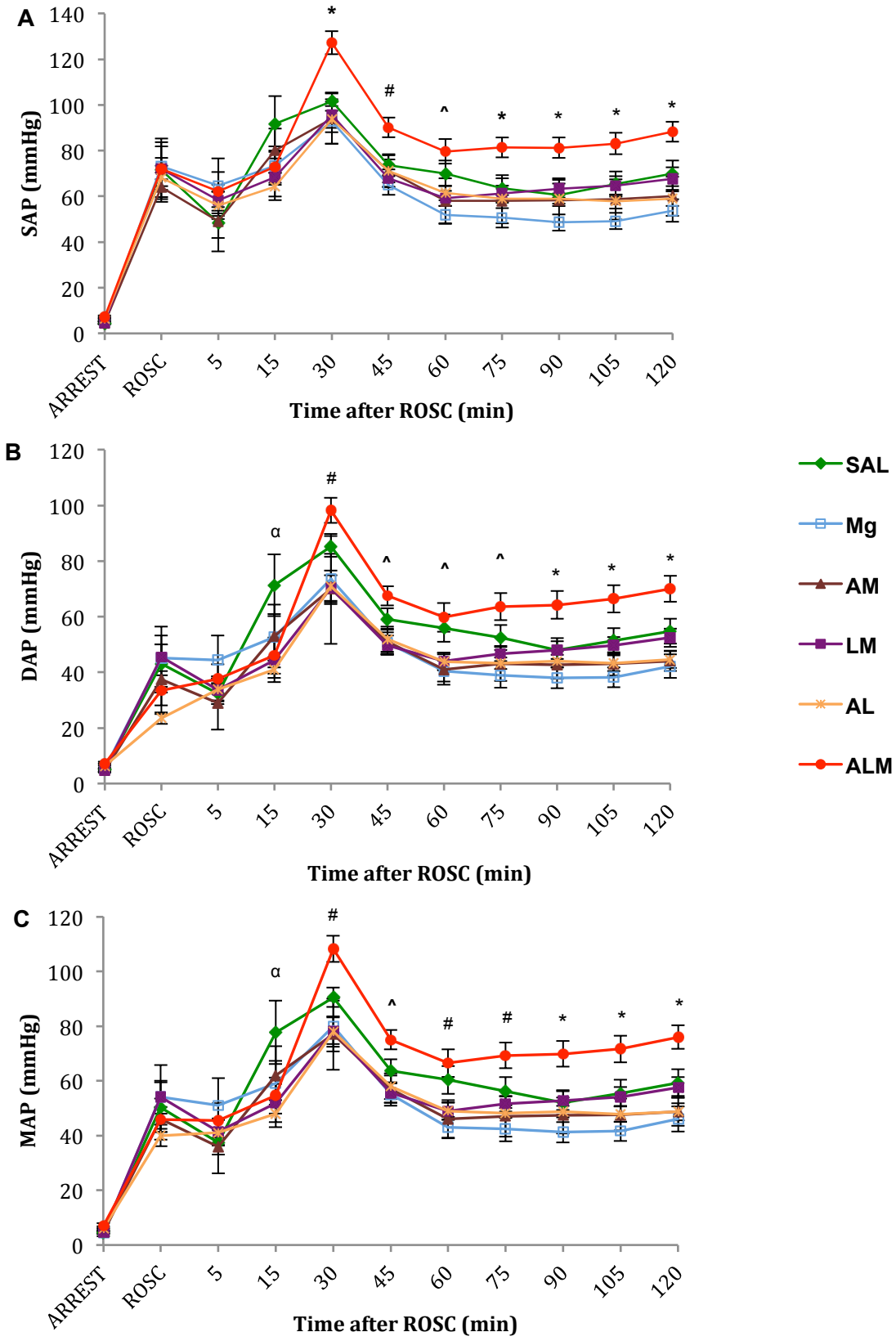


Figure 5.2 Arterial blood pressure following resuscitation among groups
A. Systolic arterial pressures (SAP), B. Diastolic arterial pressures (DAP), and C. Mean arterial pressures (MAP). Values are expressed as mean ± SEM. *p<0.05 ALM compared to all groups. #p<0.05 ALM compared to other groups except saline. ^p<0.05 ALM compared to Mg, LM and AL groups. αp<0.05 SAL compared to AL group

The heart rate (HR) following ROSC is illustrated in Figure 5.3. Immediately after ROSC, acutely low HR was experienced by rats treated with AL (47 ± 6.3 bpm), followed by AM (66 ± 4.3 bpm) and ALM (70 ± 8.1 bpm), which were approximately 47%, 66%, and 70% of HR of saline controls. However, only HR in AL alone group achieved statistical significance compared to saline, Mg and LM groups. At 5 min, the HR of AL, AM and ALM groups accelerated (88%, 99% and 172% increase, respectively) reaching a similar HR to the saline group. After 60-120 min, HR in saline control plateaued at ~200 bpm, while HRs in Mg, AM, and LM groups stabilised at ~170 bpm. At the same time, HR in AL remained constant at ~150 bpm, which was significantly lower than HR in the saline group from 30-120 min. In contrast, HR in the ALM group progressively rose above 200 bpm and became similar to that of saline after 75-120 min post ROSC. Similar to the saline group, ALM's HR was significantly higher than the AL group from 75-120 min after ROSC (Fig. 5.3).

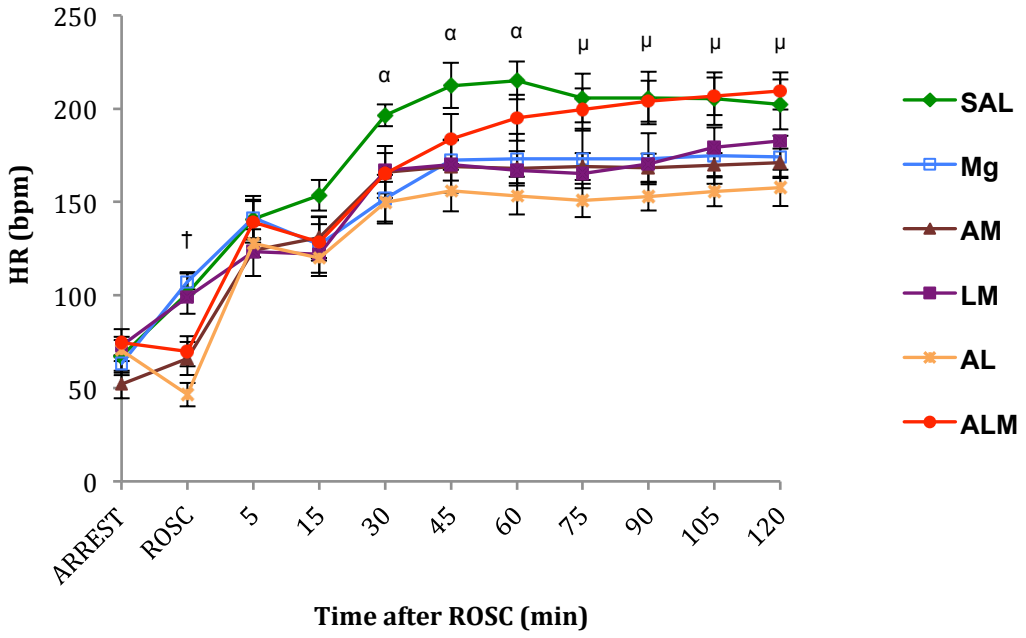


Figure 5.3 Heart rate following resuscitation among groups
Values are expressed as mean ± SEM. $^{\dagger}p<0.05$ SAL, Mg, LM compared to AL group. $^{\alpha}p<0.05$ SAL compared to AL group. $^{\mu}p<0.05$ SAL & ALM compared to AL groups

5.4.4 Incidence of arrhythmias during post-ROSC moderate hypothermia

The frequency and duration of arrhythmias experienced with different treatments are shown in Table 5.4. VT was experienced by at least one animal in all treatment groups. However, only saline- and AM-treated rats underwent VF, with an average duration of 169 sec and 75 sec, respectively. VF episodes following saline treatment were persistent leading to mortality, while a VF event self-terminated in one AM rat; however the animal eventually died within 90 min of ROSC. The frequency of VT episodes in one Mg- and two LM-treated rats were relatively higher with 9 and 11 ± 0.5 incidences per animal, respectively, and lowest in AL group with one VT lasting 2 sec occurring in one animal.

Table 5.4 Arrhythmic events experienced per animal following resuscitation with different treatments and moderate hypothermia

Group	N			Frequency of arrhythmias per animal			Duration of arrhythmias per animal (sec)		
	VT	VF	VT /VF	VT	VF	VT/VF	VT	VF	VT/VF
SAL	2	4	4	3 ± 1.0	2 ± 0.5	3 ± 1.3	11 ± 6.5	169 ± 82.3	174 ± 84.3
Mg	1	0	1	9	0	9	58	-	58
AM	2	1	3	4 ± 1.0	1	3 ± 1.2	5 ± 2.8	75	29 ± 23.3
LM	2	0	2	11 ± 0.5	0	11 ± 0.5	25 ± 7.0	-	25 ± 7.0
AL	1	0	1	1	0	1	2	-	2
ALM	1	0	1	4	0	4	8	-	8

Values are expressed as mean ± SEM. VT = ventricular tachycardia, VF = ventricular fibrillation, VT/VF = ventricular tachycardia and/or ventricular fibrillation.

5.4.5 PT and aPTT analysis

Following 120 min of moderate hypothermia, sham animals (no asphyxiation) had PT and aPTT of 29 ± 1.1 and 40 ± 3.4 sec, respectively (n=8). After 8 min asphyxial hypoxia, saline treatment led to prolonged coagulation with PT of 78 ± 26.4 sec and aPTT of 147 ± 49.7 sec. Resuscitation with Mg, AM and LM injection did not notably shorten the PT and aPTT times, and resulted prothrombin times >70 sec and aPTT longer than 130 sec. In contrast, AL injection reduced PT (by 22%) and aPTT (by 47%), but did not reach significance compared to the saline group. ALM treatment significantly reduced clotting times (PT= 30 ± 2.0 sec; aPTT= 56 ± 10.2 sec) and was comparable to sham animals.

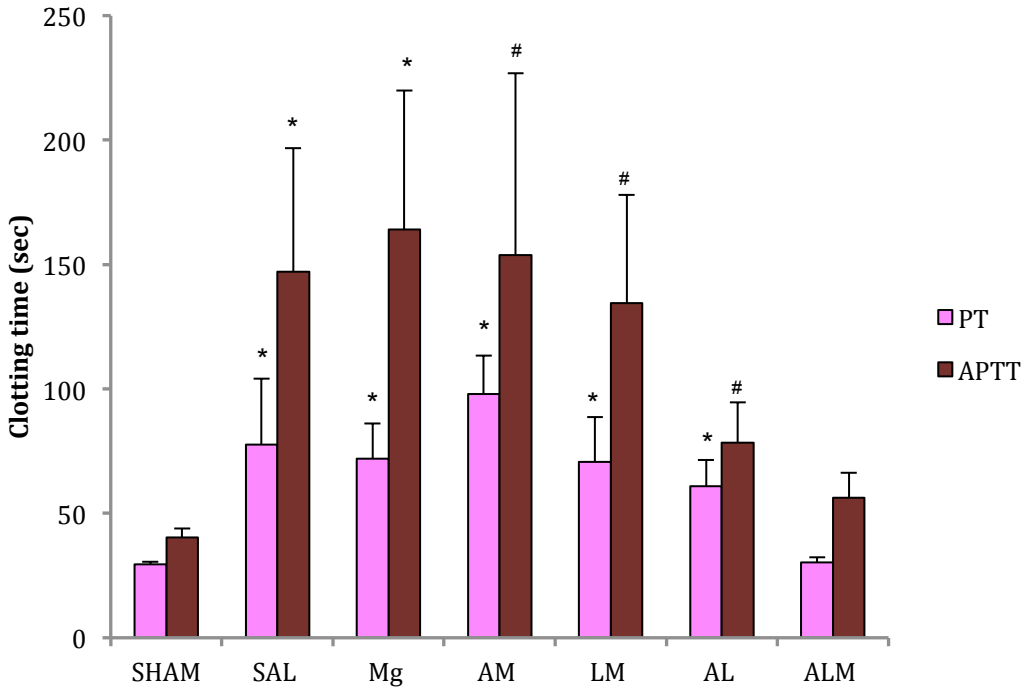


Figure 5.4 Plasma PT and aPTT among different groups following 8 min asphyxia hypoxia compared to sham animals
Values represent mean ± SEM. *p<0.05 compared to SHAM and ALM groups. #p<0.05 compared to SHAM group.

5.5 Discussion

Coagulopathy is a serious problem for cardiac arrest patients and contributes to poor survival outcomes (Adrie*, et al.*, 2005). This chapter reports 100% survival was achieved with AL and ALM resuscitation. In addition, ALM treatment significantly improved haemodynamics over 120 min post-ROSC with moderate hypothermia compared to the other groups. Both AL and ALM had no VF and less frequent VT compared to any other group. Prolonged PT and aPTT times occurred in saline controls after 120 min. Of importance, only ALM treatment significantly prevented hypocoagulopathy (particularly PT) with values not significantly different from sham animals. These findings will now be discussed.

5.5.1 ROSC attainment and haemodynamics during moderate hypothermia

AL or ALM therapy led to a significant survival benefit as at least one animal from all other treatment groups failed to achieve ROSC (Table 5.2). Interestingly, ROSC was achieved in both AL and ALM groups at 44% and 20% lower DAPs respectively than saline controls; and yet, the SAPs were similar (Fig 5.2A,B). As a result, AL and ALM animals had higher pulse pressures compared to the saline group; which were 44 ± 5.6, 38 ± 4.6 and 29 ± 2.0 mmHg, respectively (Table 5.3). The higher pulse pressures and lower DAPs are potentially resulted from lower peripheral resistance early after AL and ALM injections, possibly through adenosine A2a receptor activation. Additionally, since pulse pressure is a clinical indicator of stroke volume (Girling, 1972; Lamia*, et al.*, 2005), the data may suggest improvement in survival in AL- and ALM-treated rats is from increased stroke volume during resuscitation. Further echocardiographic and pressure-volume studies are required to test this hypothesis.

Higher pulse pressures, especially in AL animals, occurred with significantly lower heart rates compared to controls. A lower heart rate may increase diastolic filling time and diastolic volume, resulting in improved stroke volume (Girling, 1972), but as was suggested in Chapter 4, an increase in systolic ejection time may also be involved with higher stroke volumes in these low flow trauma states (Granfeldt*, et al.*, 2014). In 2013, Torgersen and colleagues examined out-of-hospital cardiac arrest victims undergoing therapeutic hypothermia, and concluded that lower heart rates at hospital admission correlated with improved outcomes (Torgersen*, et al.*, 2013). They further proposed that norepinephrine-induced increases in perfusion pressure and cardiac index in this

patient cohort may contribute to adverse neurologic outcome following out-of-hospital cardiac arrest.

In addition to ROSC achievement, the ALM treatment group showed significantly improved haemodynamics compared with AL, AM, LM and Mg alone, as well as saline controls. A significantly higher MAP in the ALM group from 30 min to 120 min for the same or lower heart rate as saline controls, may also indicate improved cardiac contractility in the ALM-treated rats. In contrast, in the AL group, the arterial pressures (SAP, DAP and MAP) were slightly lower than those of saline controls, however this did not interfere with the animal's ability to maintain ROSC.

Interestingly, small-volume 7.5% NaCl ALM, and to a lesser extent AL, has been shown to improve MAP in rats following 60 min resuscitation from severe haemorrhagic shock (Letson*, et al.*, 2012). Furthermore, Letson and colleagues showed that it was only the ALM treatment that reached significance from saline treatment (Letson*, et al.*, 2012). Additionally, in the pig model of ventricular fibrillation–induced cardiac arrest, despite a similar incidence of ROSC with and without AL, AL infusion led to ~~a~~ significantly improved early post-resuscitation cardiac function and attenuated leucocyte superoxide anion generation, without a change in post-ROSC neurological function (Granfeldt, 2012; Granfeldt*, et al.*, 2013).

5.5.2 Role of magnesium in improved haemodynamics

Improved haemodynamics in the ALM group during moderate hypothermia was due to the addition of Mg^{2+} to the AL solution. While the mechanism for improvement is not known at present, there is a vast array of literature showing that the addition of Mg^{2+} improves cardiac function after cardiac surgery (Caspi*, et al.*, 1995). Possible mechanisms include: 1) improved Ca^{2+} handling and pro-survival Ca^{2+} signalling pathways (Agus & Agus, 2001); 2) improved mitochondrial oxidative phosphorylation and ATP replenishment (Saris*, et al.*, 2000); 3) improved regulation of Na^{+} and K^{+} transport across cell membranes and electrical stability (Bara*, et al.*, 1993; Mubagwa*, et al.*, 2007); 4) inverse relationship between arterial blood pressure and the level of ionized intracellular and plasma Mg^{2+} (Altura*, et al.*, 1993); 5) a reduced energy demand from magnesium's 'anaesthetic-like' properties (Soave*, et al.*, 2009), and 6) anti-arrhythmic properties (Geiger & Wanner, 2012; Khan*, et al.*, 2013).

However, the addition of Mg to AL is a very different story from Mg itself as the administration of Mg^{2+} alone in this chapter produced the lowest arterial pressures after 30 min of ROSC (Fig 5.2). This was not the case for the ALM treatment group. The mechanisms of adding Mg to AL to increase the incidence of ROSC and improve post-ROSC haemodynamics requires further investigation.

5.5.3 ALM and AL treatments led to no VF during moderate hypothermia

In the present study, and as in published studies on AL and ALM (Canyon & Dobson, 2004; Letson & Dobson, 2011a, 2011b), AL- and ALM-treated animals had very few severe arrhythmias during hypothermia (Chapter 4 and 5). As indicated in previous chapters, the electrical stability effects of AL or ALM are not known in global hypoxia/ischaemia as seen in the rat model. In 2010, Dobson proposed that the anti-arrhythmic properties of AL or ALM may arise from the drug's ability to defend a more polarised diastolic resting membrane potential thus preventing the heart muscle from large falls in depolarization into the "trigger" zone of about -60 mV that is thought to initiate ventricular arrhythmias (Dobson, 2010).

It is well known that moderate hypothermia leads to increased arrhythmias from ventricular repolarization lengthening or refractoriness (Luscombe & Andrzejowski, 2006). In the present study, 25% and 50% of the saline rats experienced VT and VF, respectively. In 2011, Nielsen et al. reported that hypothermia led to an increased incidence of arrhythmias of about 18% compared to non-hypothermic patients (Nielsen*, et al.*, 2011). However, in addition to moderate hypothermia many other factors could contribute to increased cardiac excitability including post-arrest electrolyte disorder, acidosis, and hypoxia (Luqman*, et al.*, 2007), as well as chest compressions during resuscitation (Berdowski*, et al.*, 2010; Osorio*, et al.*, 2008).

5.5.4 Coagulation status following resuscitation and moderate hypothermia with different drug treatments

The presence of acute coagulopathy following cardiac arrest has been demonstrated in clinical and animal studies (Adrie*, et al.*, 2005; Bottiger*, et al.*, 1995; Gando*, et al.*, 1997; Gando*, et al.*, 1999; Johansson*, et al.*, 2003). The present study shows that after asphyxial-induced cardiac arrest, PT and aPTT in control group increased 2.5 and 3.5 times, respectively, at two hour post resuscitation (Fig. 5.4). Extended PT and aPTT is an indication of dysfunctional extrinsic and intrinsic coagulation pathways, respectively.

The impaired coagulation appears not to have resulted from induced hypothermia as sham hypothermic animals presented similar PT and aPTT times to healthy normothermic rats (data not shown). In agreement with the present data, prolongation of PT and aPTT values have been found in out-of-hospital cardiac arrest patients and are associated with increased protein C activity during hospital admission (Adrie*, et al.*, 2005). Brohi and colleagues argue that the main factor in producing a hypocoagulable state is hypoperfusion associated with trauma (Brohi*, et al.*, 2008).

The clinical importance of coagulopathy following arrest has been described previously and is strongly associated with poorer prognosis (Kim*, et al.*, 2013) and increased mortality following cardiac arrest (Adrie*, et al.*, 2004). Apparently, cardiac arrest-induced coagulopathy arises from ischaemia/reperfusion-induced endothelial dysfunction (Adams, 2006), and interestingly, the degree of coagulopathy may vary depending on the stage of perfusion (White, et al., 2011). White and colleagues (2011) showed coagulation profiles may change during VF (arrest), CPR and post-ROSC (White*, et al.*, 2011). Slightly reduced reaction times (faster clotting time) and increased clotting firmness were observed during arrest, whereas, increased kinetic times (clot propagation time) and reduced firmness were shown during CPR and post-ROSC. This present study confirmed this finding by showing increased clotting times (PT and aPTT) in control animals two hours post-ROSC.

In this study, it was shown that neither Mg, AM, LM nor AL treatment was able to prevent the hypocoagulopathy at 120 min following asphyxial-induced cardiac arrest. In contrast, ALM injection resulted in significantly shorter clotting times near the PT and, to a lesser extent, aPTT values of sham animals. A similar result with ALM has been demonstrated in the rat model of haemorrhagic shock (Letson*, et al.*, 2012). Letson and co-workers (2012) showed that after 60 min of shock (40% blood loss), PT and aPTT times increased 10- to 13-fold from baseline, and after 60 min resuscitation with small-volume 7.5% NaCl ALM both PT and aPTT were corrected to baseline values (Letson*, et al.*, 2012).

The mechanism underlying the coagulation benefit with ALM is not fully understood, but may involve: 1) improved systemic perfusion, since the presence of hypoperfusion is correlated with acute coagulopathy in trauma and cardiac arrest patients (Brohi*, et al.*, 2008; Viersen*, et al.*, 2012); and/or 2) lower O_2 consumption, lactate production and ion H^+ release during arrest and reperfusion. Viersen et al. (2012) reported that

acute coagulation in cardiac arrest victims is strongly associated with reduced pO_2, increased lactate, and acidosis.

5.5.5 Limitation of the study

This present study showed that ALM injection with hypothermic resuscitation followed by post-ROSC moderate hypothermia (28-32^{o}C) led 100% successful ROSC and significantly improved post-ROSC haemodynamics without significant arrhythmias. However, this study did not directly measure the cardiac function parameters such as stroke volume, cardiac output, dP/dTmin, dP/dTmax, and end diastolic volume/pressure. Future study is warranted to confirm ALM effects on cardiac function parameters following asphyxial-induced cardiac arrest and moderate hypothermia.

Another limitation is that post-cardiac arrest coagulation status were measured using conventional coagulometry (PT and aPTT tests). However, these tests analyse platelet-poor plasma and thus cannot measure the factual rate of clot formation, clot amplitude, or degree of fibrinolysis (Park*, et al.*, 2009). Further assessment of coagulation status with thromboelastography or rotational thromboelastometry can provide better indication of the presence of hypocoagulopathy and/or hyperfibrinolysis post cardiac arrest (Park*, et al.*, 2009; Schöchl*, et al.*, 2013; Viersen*, et al.*, 2012).

5.6 Conclusion

In summation, although both AL and ALM administration during hypothermic resuscitation resulted in 100% ROSC attainment and sustainability with no fatal arrhythmias, only ALM treatment led to significantly improved haemodynamics following ROSC during moderate hypothermia. Furthermore, asphyxial-induced cardiac arrest with post-ROSC moderate hypothermia was found to induce abnormal coagulation shown by extended PT and aPTT at two hours after ROSC. The abnormal clotting times were prevented with a small bolus of ALM during resuscitation.

CHAPTER 6. EFFECT OF ALM ON POST RESUSCITATION HAEMODYNAMICS, COAGULOPATHY AND EARLY BRAIN HISTOPATHOLOGY FOLLOWING INTRA-ARREST HYPOTHERMIA AND ACTIVE POST-ROSC REWARMING[2]

6.1 Introduction

In Chapter 5, moderate hypothermia (28-33^{o}C) was used during the intra-arrest and 120 min post-resuscitation period to study the effect of different drug treatments on ROSC, haemodynamics and coagulopathy during hypothermia. It was found that both AL and ALM treatment led to 100% successful ROSC, improved haemodynamics 90-120 min post ROSC compared with any other group, and no VF. In addition, ALM, but not AL, prevented hypocoagulopathy (PT and aPTT) after two hours of ROSC.

The present chapter will investigate the effect of intra-arrest hypothermia (~33^{o}C) followed by active rewarming immediately following cardiac arrest (33-37°C). Thus, the protocol differs from Chapter 5 in that moderate hypothermia is not maintained post-resuscitation. The effects of ALM on ROSC achievement, post-ROSC haemodynamics and a more detailed coagulation assessment will be undertaken using state-of-the-art whole blood viscoelastic ROTEM analysis. Early brain histology will be examined to see if ALM demonstrates any neuroprotective effects.

6.2 Aims

To examine the effect of ALM on ROSC achievements, post-ROSC haemodynamics, coagulation profile (ROTEM and plasmatic PT/aPTT analyses) after two hours of ROSC following asphyxial-induced cardiac arrest with intra-arrest cooling (~33^{o}C) and active rewarming (to 37^{o}C) post resuscitation. Early neurohistological changes were also assessed in this study.

[2]Some results in Chapter 6 have been published in *Shock.* 2013 Sep;40(3):222-32 (see detail on page vii)

6.3 Experimental Protocols

6.3.1 Experimental design

Animal housing, feeding and water regimes, ethics approvals, methods of anaesthesia and surgical protocols are described in Chapter 2. Non-heparinised, male Sprague-Dawley rats (400-500 g, n= 39) were randomly assigned to one of two groups: 1) SAL (n=12) and 2) ALM (n=10) (Fig. 6.1). The number of animals was increased in this chapter to increase the power of statistical analysis. The doses used for this study are the same as in Chapter 5 and shown in Fig 6.1.

Rats were anaesthetised, surgically prepared and stabilised for 10-15 minutes before collecting the baseline haemodynamic data as described in Chapter 2. Cardiac arrest was induced by turning off and clamping the tracheotomy tube from ventilator for eight minutes. Surface cooling (ice-cold pack and ethanol spray) was applied during asphyxiation to target 33^{o}C at the time of resuscitation. The rats that achieved ROSC were immediately rewarmed under a heating lamp (50-W halogen). Active or fast rewarming is avoided in clinical settings since it may induce haemodynamic, coagulation and neurological disturbance (Bouwes*, et al.*, 2012). However, it was of interest in the present chapter to see if active rewarming reduced ALM's effectiveness to resuscitate and correct coagulopathy.

Resuscitation was commenced by declamping the ventilation tube and injecting 0.5 ml bolus of drugs i.v. 10 sec before restarting the ventilator (at the same stroke rate of 95-100 strokes/min^{-1}), followed by chest compressions (300/min) for 60 sec. If ROSC was not achieved, another 30 sec of compressions were performed, followed by a third and last attempt if that effort failed to produce ROSC. No further compressions or drug treatment was administered during the two hour recovery period. Heart rate and arterial pressures (SAP, DAP and MAP) and ECG stability under active rewarming were continuously monitored for 120 min for each treatment group.

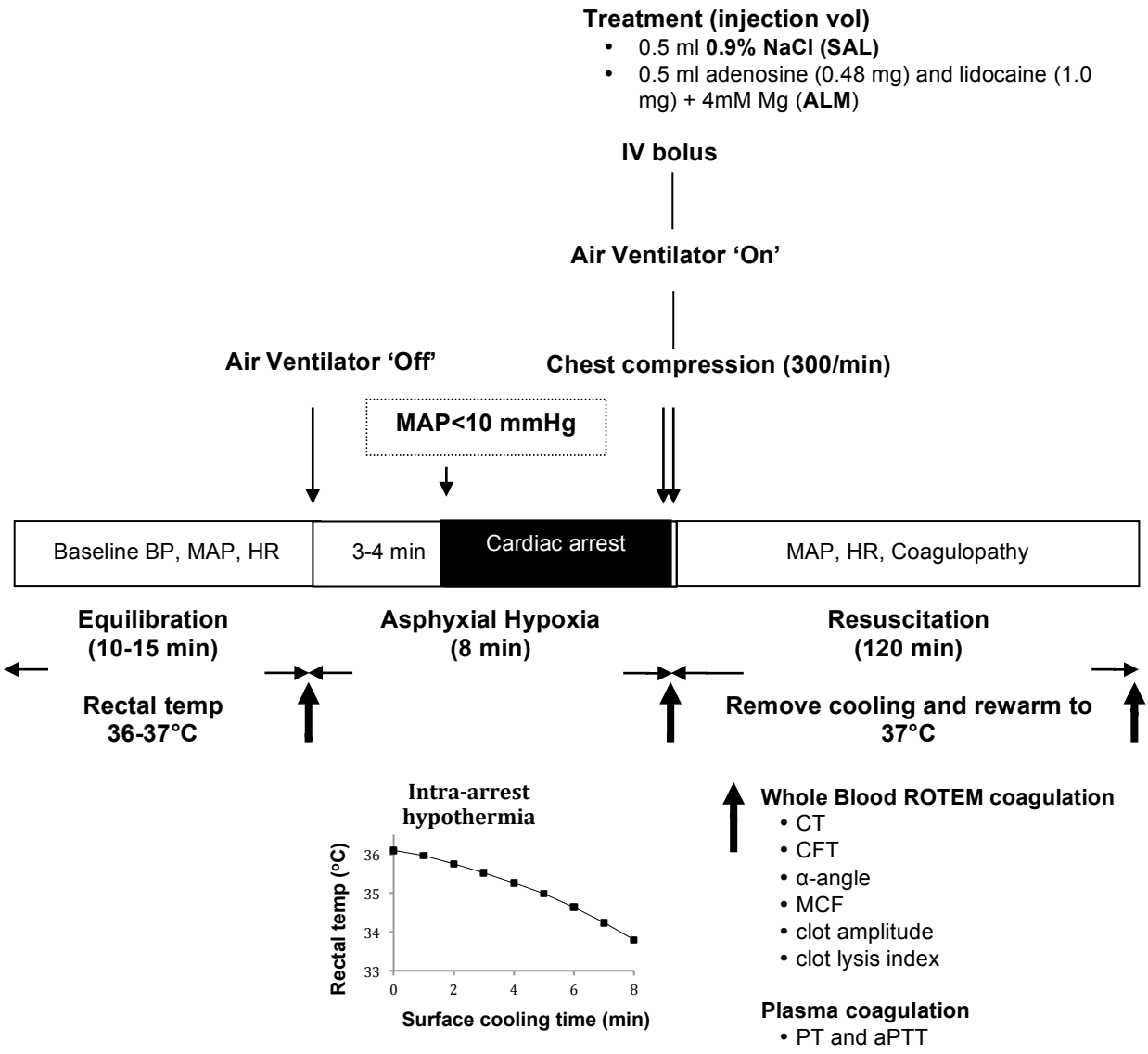


Figure 6.1 A broad schematic of the *in vivo* non-heparinised rat model of 8 min asphyxial cardiac arrest
Therapeutic mild hypothermia was induced using an ice-pack and ethanol spray during the intra-arrest period (inset). The ice-pack was removed before drug administration, chest compressions and ROSC, then the animal was actively rewarmed (see experimental design section). Blood samples were withdrawn at baseline, during 8-min asphyxia, and after 120 min post ROSC.

6.3.2 Coagulation assays

Blood samples were taken during baseline (baseline), at the end of 8 min asphyxial hypoxia (cardiac arrest), and two hours after ROSC, or in those animals that failed to attain ROSC, in the first 2-5 min of attempts. Coagulation profile was measured using whole blood thromboelastometry (ROTEM) including EXTEM, INTEM, and FIBTEM tests as described in Chapter 2.

The following parameters were measured: Clotting time (CT), the time from start of measurement until a clot amplitude of 2 mm; clot formation time (CFT), the time from end of CT until a clot firmness of 20 mm; and maximum clot firmness (MCF), the final strength of the clot in mm arising from the interaction of fibrin and activated by platelets and Factor XIII. The alpha angle (α) was also measured and represents the angle between baseline and a tangent at the maximum clot slope and clot amplitude (amplitude at 5 to 30 min) in mm over a 30 min period (Fig. 6.2). The lysis index (LI, %) was estimated as the ratio of clot firmness (amplitude at 30 or 60 min) divided by MCF times 100. LI is an estimate of fibrinolysis, and hyperfibrinolysis was defined as estimated percent lysis ≥15% at 60 min (Lang*, et al.*, 2005). Maximum clot elasticity (MCE), an indicator of clot strength, was calculated from MCE = (MCF x 100)/(100 - MCF) (Lang*, et al.*, 2009). The platelet contribution to clot strength was calculated from $MCE_{platelet} = MCE_{EXTEM} – MCE_{FIBTEM}$. The ROTEM trace featuring these parameters is illustrated in Fig. 6.2.

In addition, PT and aPTT analyses were performed with the remaining blood samples from ROTEM tests using the protocols described in Chapter 2.

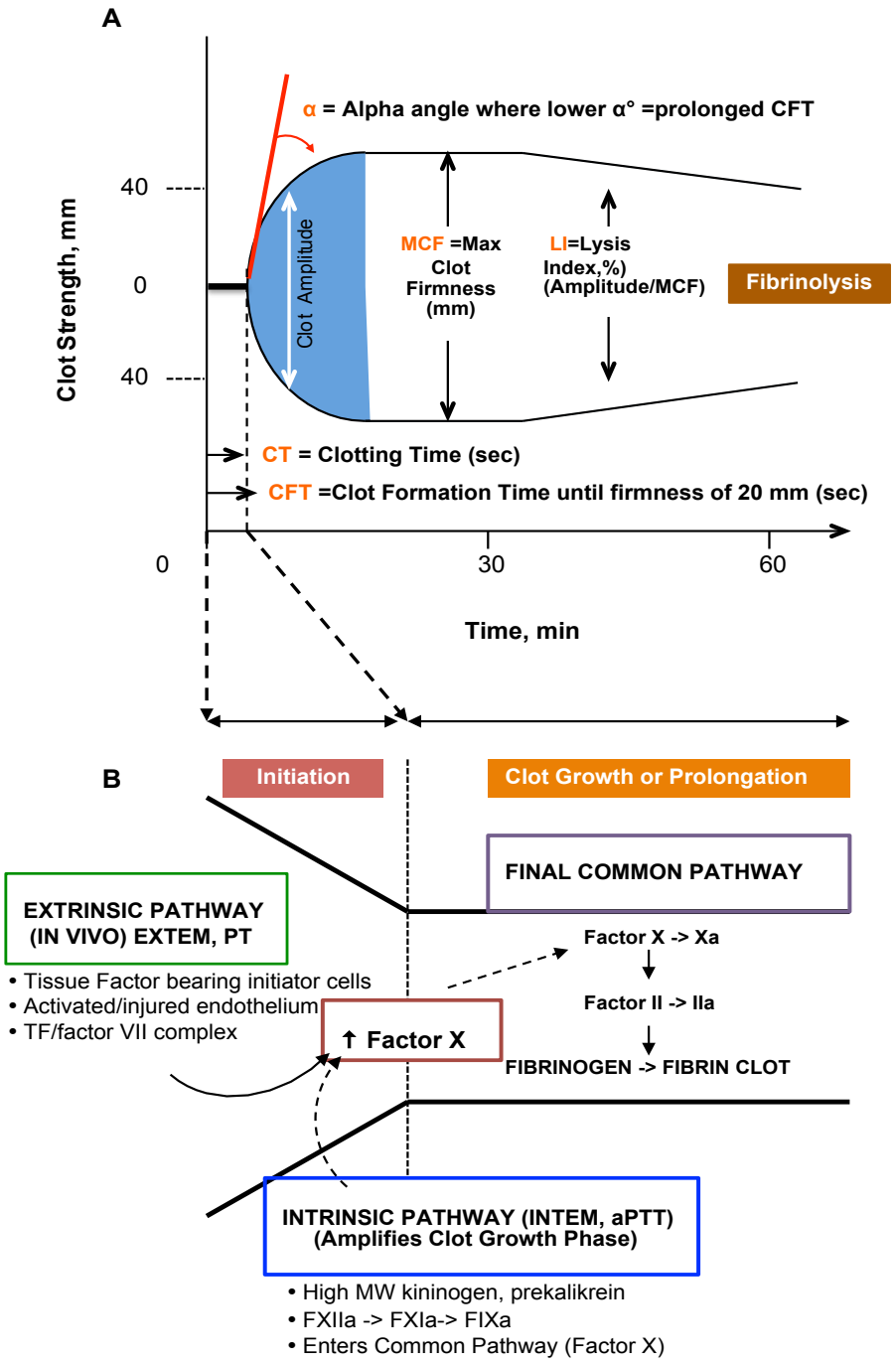


Figure 6.2 Representation of a ROTEM trace
Key coagulation parameters and definitions are described showing the initiation, formation and growth phases of clot coagulation and breakdown (retraction).

6.3.3 Neurological study

Neuronal damage categories and quantification methods can be found in Chapter 2. The number of injured neurons was counted in two different areas: hippocampal CA1 and neocortex (see Fig. 2.7). Damaged neurons were identified by their dark shrunken morphology, strong eosinophilic, pyknotic nuclei and irregularly distributed cytoplasm (see Fig. 2.8). Only neurons that had no recognisable nucleus or low-nuclear-cytoplasmic border were counted. The number of ischaemic neurons presented for each rat was the average of the total number of damaged neurons in both hemispheres (either CA1 or cortical region) in two subsequent sections. Three additional rats underwent surgical protocol, without asphyxial hypoxia or drug infusion, and were sacrificed as sham controls.

6.3.4 Statistical analysis

Statistical analysis was performed using SPSS statistical package (PASW statistic 18). All values are expressed as mean ± SEM. The survival between groups was compared by plotting *Kaplan-Meier* survival curves with log-rank test. *Kolmogorov-Smirnov* analysis was applied to test for normal distribution. Normally distributed data were analysed using one-way analysis of variance with *Tukey's* post-hoc test for multiple comparisons, whereas haemodynamic data comparing saline and ALM groups were evaluated with student T-test. Data that were not normally distributed were analysed with *Kruskal-Wallis* test and *Mann-Whitney U* test. The pairwise comparisons were applied only when *Kruskal-Wallis* test was significant. Statistical significance was defined as $p<0.05$.

6.4 Results

6.4.1. Baseline haemodynamics, cooling rate and cardiac arrest time

No significant differences in baseline haemodynamics were found. Systolic arterial pressures ranged from 127 to 138 mmHg, DAPs ranged from 117 to 127 mmHg, MAPs ranged from 120 to 131 mmHg, HR from 372 to 401 beats/min, and rectal temperature (RT) from 36.7 to 37.0^{o}C. During cooling, RT fell at a rate of ~0.1^{o}C/min for the first 4 min and ~0.2 ^{o}C/min during the second 4 min (i.e. RT was 36^{o}C at the onset of cardiac arrest and ~33 ^{o}C at 8 min; Fig. 6.1). Time to cardiac arrest and duration were 213 ± 16 and 267 ± 6 sec for saline group (n=12) and 208 ± 14 and 257 ± 9 sec for ALM treatment group (n=10)

Table 6.1 Baseline haemodynamics, rectal temperature, time to and duration of cardiac arrest among treatment groups

Group	n	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (bpm)	Temp (^{o}C)	Time to CA (sec)	Duration of CA (sec)
Baseline	10	136 ± 6.4	117 ± 5.1	124 ± 5.6	401 ± 12.8	37.1 ± 0.1	NA	NA
CA	10	138 ± 5.5	127 ± 5.5	131 ± 5.5	392 ± 11.1	36.7 ± 0.1	218 ± 8.1	260 ± 5.9
SAL	12	127 ± 4.3	117 ± 3.8	120 ± 3.9	372 ± 8.0	37.0 ± 0.1	213 ± 16.1	267 ± 5.8
ALM	10	132 ± 4.3	121 ± 3.9	124 ± 4.0	373 ± 4.5	37.0 ± 0.1	208 ± 14.3	257 ± 9.1

Values are expressed as mean ± SEM. SAP = systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, HR = heart rate, Temp = rectal temperature, Time to CA = time to cardiac arrest from the initiation of asphyxia to the onset of cardiac arrest (MAP <10 mmHg), Duration of CA = time from cardiac arrest to resuscitation attempt. No significant differences found between groups.

6.4.2 ROSC achievements and Kaplan-Meier survival distribution

Following three attempts of chest compressions, 33% of saline controls (4 of 12) failed to attain ROSC from refractory VF, and these animals were included only in ROTEM data analysis (Table 6.2). All of the ALM rats attained ROSC after chest compressions (10 of 10), and this difference was significant using a *Kaplan-Meier* distribution (Fig 6.3.)

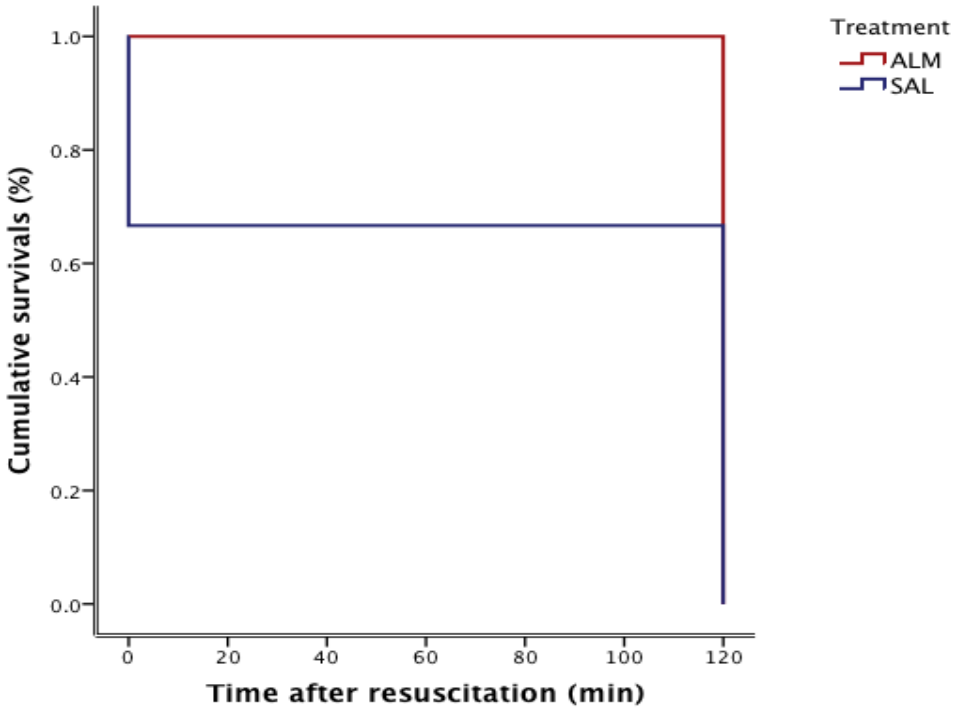


Figure 6.3 *Kaplan-Meier* test of survival distributions (achievement of ROSC) in control and ALM-treated rats following chest compressions
Log-rank (Mantel-cox) $\chi 2$=3.889 (P= 0.049)

6.4.3 Post-resuscitation haemodynamics, heart rate, and rectal temperature

During the first 5 min, MAP in the ALM group was significantly lower than that in controls (36 vs 55 mmHg at t=0, and 42 vs 73 mmHg at 5 min) (Fig. 6.4A). At 15 min, MAP in the ALM group sharply increased 2.6 fold and controls 1.4 fold to ~100 mmHg. Then, in both groups, MAP decreased to ~60 mmHg at 45 min. From 45 to 120 min, the ALM group generated higher MAPs than controls, with significant differences at 75 min (74 vs 62 mmHg) and at 120 min (81 vs 72 mmHg) (Fig 6.4A). Higher MAP in the

ALM group at 75 and 120 min was due to significantly higher systolic and diastolic arterial pressures (data not shown).

Heart rate was significantly lower in the ALM group at t = 0 to 5 min (Fig. 6.4B). There were no significant differences between 15 and 120 min between the two groups. During active external rewarming from t = 0, RT did not increase significantly in either group until 15 min after ROSC, after which it increased at rate of ~0.058 ^{o}C/min to 75 min and ~0.022 ^{o}C/min between 75 to 120 min (Fig. 6.4C).

6.4.4 ROTEM (whole blood) coagulation parameters

6.4.4.1 Baseline and cardiac arrest (CA)

Baseline EXTEM, INTEM, and FIBTEM values for CT, CFT, α-angle, MCF, clot amplitudes (A5-30), LI, and MCE are shown in Table 6.2, Fig. 6.5 A,B, and Fig. 6.6 A,B. Baseline clot amplitudes ranged from 65 mm (A5) to 73 mm (A30), and LI(30-60 min) from 87 to 100% (Table 6.2). After 8 min of cardiac arrest (CA group), EXTEM and INTEM values for CT, CFT, α-angle, and MCF were not significantly different from baseline, nor was the LI (LI30-60) (Table 6.2, Fig. 6.5 A,B, and Fig. 6.6 A,B). FIBTEM CT was significantly longer (47 vs 36 sec), and FIBTEM LI30-60 was significantly reduced (95 vs 100%) compared to baseline immediately after CA. EXTEM and INTEM MCE and the platelet contribution ($MCE_{platelet}$) were ~10% lower after 8 min CA compared with baseline but were not significantly different (Table 6.2). Baseline $MCE_{platelet}$ values for baseline and arrest were 279 ± 12 and 247 ± 14, respectively.

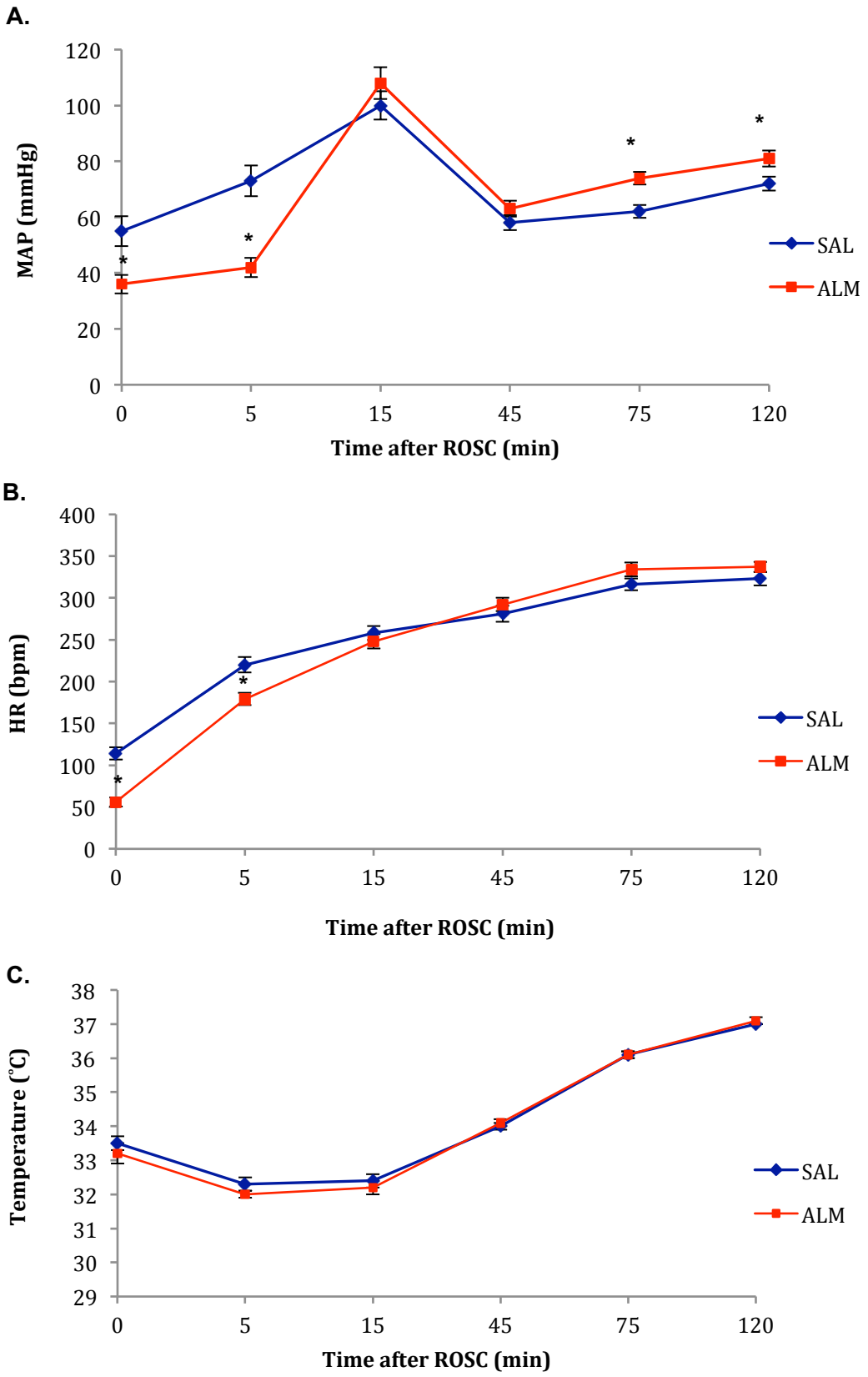


Figure 6.4 Changes in (A) MAP, (B) HR, and (C) RT in saline and ALM treated rats monitored for 120 min after ROSC
Values represent mean ± SEM. *p<0.05 compared to saline group

Table 6.2 Effect of cardiac arrest, small bolus of 0.9% NaCl (Saline) and ALM on ROTEM coagulation parameters following 8 min of asphyxial cardiac arrest

Treatment	Test	Clot Time	Clot Formation Time		Max Clot Firmness	Clot Amplitude						Lysis Index			
(n)		CT (sec)	CFT (sec)	α (°)	MCF (mm)	A5 (mm)	A10 (mm)	A15 (mm)	A20 (mm)	A25 (mm)	A30 (mm)	LI30 (%)	LI45 (%)	LI60 (%)	MCE
Baseline (10)	EXTEM	37 ± 2.1	33 ± 2.0	84 ± 0.4	75 ± 0.8	65 ± 1.3	71 ± 1.1	74 ± 0.8	74 ± 0.7	74 ± 1.1	73 ± 1.3	98 ± 0.8	92 ± 2.7	87 ± 3.3	297 ± 12.1
	INTEM	109 ± 10.8	32 ± 2.5	84 ± 0.5	76 ± 1.1	66 ± 0.9	72 ± 0.9	74 ± 1.0	76 ± 1.2	76 ± 1.3	76 ± 1.3	100 ± 0.3	99 ± 0.5	98 ± 0.7	329 ± 16.8
	FIBTEM	36 ± 1.8	NA	79 ± 1.1	15 ± 0.6	14 ± 0.7	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.5	100 ± 0.0	100 ± 0.0	100 ± 0.0	18 ± 0.8
Cardiac arrest (t=8 min)															
CA (7)	EXTEM	43 ± 3.3	30 ± 1.0	84 ± 0.3	74 ± 2.0	69 ± 1.2	71 ± 1.4	68 ± 2.8	65 ± 4.2*	62 ± 4.8$^{\#}$	59 ± 5.3$^{\#}$	82 ± 6.6$^{¥}$	69 ± 9.3$^{¥}$	61 ± 10.3$^{¥}$	266 ± 15.0
	INTEM	121 ± 13.0	33 ± 4.3	83 ± 0.8	75 ± 0.7	66 ± 1.3	72 ± 1.1	74 ± 1.0	75 ± 0.8	75 ± 0.8	75 ± 0.9	100 ± 0.2	97 ± 1.0^	95 ± 1.4^	306 ± 12.5
	FIBTEM	47 ± 3.8*	NA	74 ± 2.1	16 ± 0.9	15 ± 1.1	15 ± 0.9	15 ± 0.8	15 ± 0.9	15 ± 0.8	15 ± 0.9	94 ± 2.2^{ß}	95 ± 2.1$^{\#}$	95 ± 1.4$^{\#}$	19 ± 2.1
ROSC (t=120 min)															
Saline (8)	EXTEM	71 ± 12.2$^{¥}$	68 ± 20.1$^{¥}$	77 ± 3.4$^{¥}$	68 ± 3.4	55 ± 4.4$^{¥}$	63 ± 4.4$^{\#}$	66 ± 4.2$^{\#}$	67 ± 3.9*	67 ± 3.8*	67 ± 3.4*	98 ± 1.0	91 ± 3.5	87 ± 4.4	231 ± 22.4
	INTEM	212 ± 50.9*	110±33.8^{µ}	66 ± 8.7^{µ}	64 ± 6.7*	45 ± 8.0^{µ}	55 ± 7.6^{µ}	59 ± 7.5^{µ}	61 ± 7.3^{µ}	63 ± 7.3*	63 ± 7.2*	100 ± 0.0	100 ± 0.2	99 ± 0.6	222±37.5*
	FIBTEM	91 ± 29.9*	NA	77 ± 1.1	14 ± 1.9	12 ± 1.7	12 ± 1.8	13 ± 1.9	13 ± 1.9	14 ± 1.9	14 ± 1.8	100 ± 0.4	100 ± 0.4	99 ± 0.6	17 ± 2.7
ROSC (t=120 min)															
ALM (10)	EXTEM	41 ± 4.8	35 ± 2.6	83 ± 0.6	75 ± 1.3	65 ± 1.4	71 ± 1.2	73 ± 1.1	73 ± 1.5	73 ± 1.9	72 ± 2.4	96 ± 2.3	91 ± 3.2	87 ± 3.5	302 ± 22.5
	INTEM	197 ± 44.0	56 ± 13.3	79 ± 2.3	75 ± 0.8	60 ± 2.9	68 ± 1.9	72 ± 1.4	73 ± 1.2	74 ± 1.2	75 ± 0.9	100 ± 0.0	100 ± 0.1	99 ± 0.5	304 ± 14.3
	FIBTEM	44 ± 5.2	NA	75 ± 1.8	14 ± 0.9	13 ± 0.9	13 ± 0.9	14 ± 0.9	14 ± 0.9	14 ± 1.0	15 ± 0.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	17 ± 1.3
Without ROSC (t<5min)															
Saline (4)	EXTEM	43 ± 3.2	36 ± 2.6$^{$}$	83 ± 0.6	71 ± 1.4	63 ± 2.0	69 ± 1.7	69 ± 2.1	67 ± 3.0	64 ± 3.5*	63 ± 3.5*	89 ± 3.4†	77 ± 4.7†	69 ± 7.0$^{\#}$	241 ± 17.4
	INTEM	175 ± 28.6*	49 ± 8.7	80 ± 1.8^{µ}	73 ± 1.2*	60 ± 2.7^{µ}	68 ± 1.9^{µ}	71 ± 1.6*	72 ± 1.5*	72 ± 1.5*	72 ± 1.8*	97 ± 1.9$^{∞}$	95 ± 3.5	93 ± 5.4	280 ± 16.7
	FIBTEM	42 ± 1.3*	NA	73 ± 2.8	14 ± 2.1	13 ± 2.4	13 ± 2.1	13 ± 2.1	14 ± 1.9	14 ± 1.9	14 ± 1.9	100 ± 0.0	99 ± 1.5	97 ± 2.1$^{\#}$	16 ± 3.0

Data also includes controls that did not achieve ROSC. Values are expressed as mean ± SEM. *p<0.05 compared to baseline only. $^{\#}$p<0.05 compared to baseline and ALM groups. $^{¥}$p<0.05 compared to all other groups except saline without ROSC. ^p<0.05 compared to saline and ALM group. ^{µ}p<0.05 compared to baseline and cardiac arrest groups. $^{$}$p<0.05 compared to cardiac arrest group only. ^{ß}p<0.05 compared to all other groups. †p<0.05 compared to all other groups except cardiac arrest group. $^{∞}$p<0.05 compared ALM group only.

6.4.4.2 Saline and ALM group (after 120 min ROSC)

At 120 min after ROSC, EXTEM and INTEM values for CT and CFT for saline controls significantly increased twofold to threefold, and α-angle as well as clot amplitudes were significantly lower with no change in LI compared with baseline values (Table 6.2, Fig. 6.5 C,D, and Fig. 6.6 C,D). Saline control clot firmness at 120 min was significantly lower than baseline (64 vs 76 mm) (Table 6.2; Fig. 6.5 C,D). In contrast, EXTEM values for CT, CFT, α-angle, MCF, clot amplitudes, and LI for the ALM group rats were not significantly different from baseline (Table 6.2; Fig 6.5 E,F, and Fig. 6.6 C,D). INTEM CTs for saline controls and ALM rats did not differ at 212 and 197 sec, respectively. However, CFT was nearly half for the ALM-treated rats compared with controls (56 vs 110 sec) for a similar α-angle, and MCF was higher by 20% (75 mm vs 64 mm). EXTEM MCF fell in saline controls, decreased by 22% compared with baseline, and INTEM MCE fell by over 30% ($p<0.05$) with no change in FIBTEM MCE (Table 6.2). ALM EXTEM and INTEM MCE were comparable to baseline. Saline rats $MCE_{platelet}$ decreased by 23% from baseline, while that of ALM was significantly higher at 285 ± 21.

6.4.4.3 Saline controls that failed to attain ROSC (t=0-5 min)

For saline controls that failed to attain ROSC (n=4) EXTEM values for CT, CFT, α-angle, and MCF were 43 sec, 36 sec, 83^{o}, and 71 mm; while INTEM values were 175 sec ($p<0.05$), 49 sec, 80^{o} ($p<0.05$), and 73 mm ($p<0.05$), respectively. EXTEM A25-A30 and EXTEM LI30-LI60 were significantly lower than baseline (Table 6.2; Figs. 6.5G,H and 6.6A,B). INTEM amplitude (A5-A30) was also lower; however, in contrast to EXTEM, there was no change in clot LI (Table 6.2). FIBTEM CT for non-ROSC saline rats was also significantly higher than baseline (Table 6.2). EXTEM and INTEM MCE in the four non-survivors decreased by 15% to 19% compared with baseline (Table 6.2), and $MCE_{platelet}$ was 224 ± 17 which was 20% lower than baseline. Interestingly, the decrease was similar to that seen in saline rats with ROSC

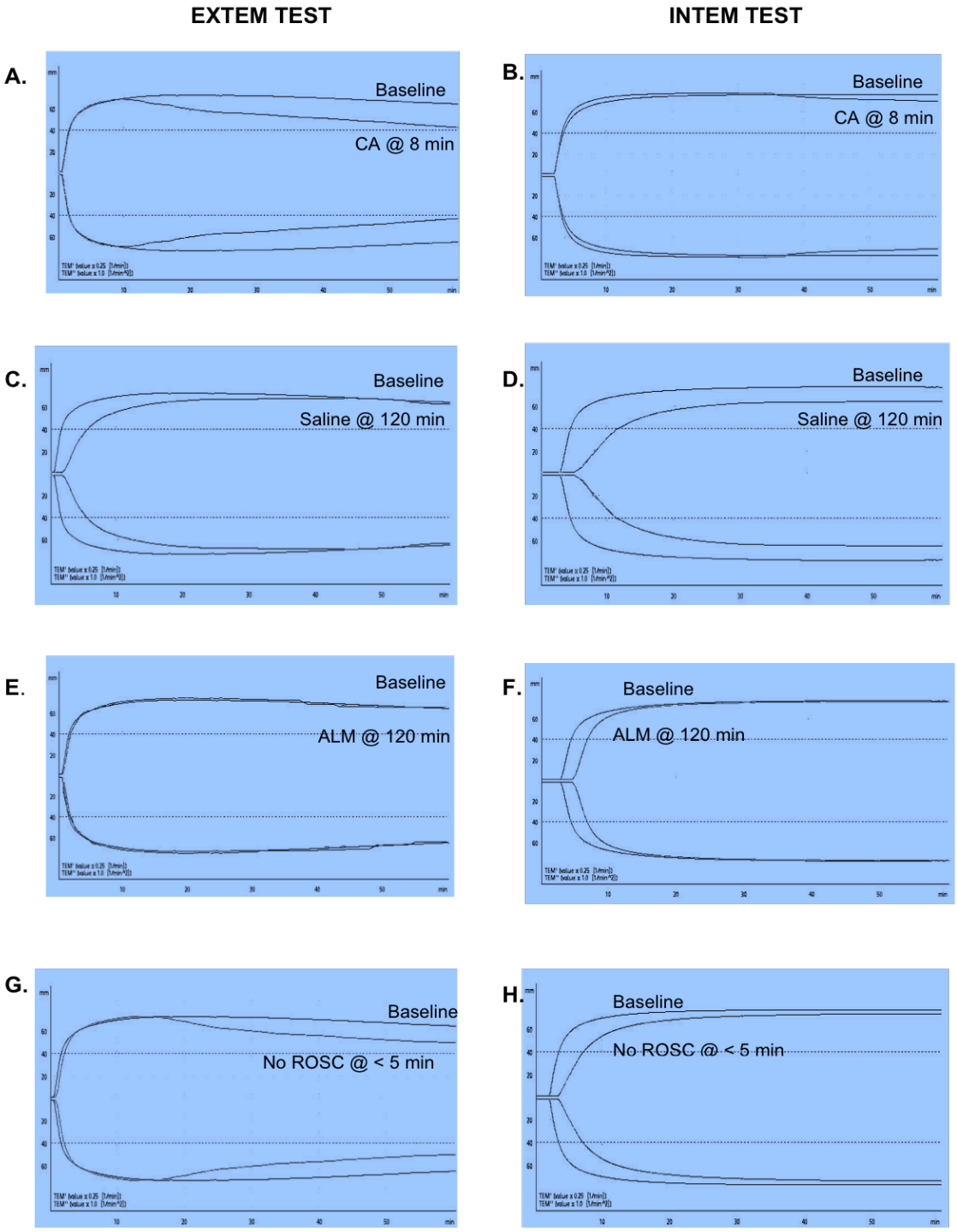


Figure 6.5 Representative ROTEM traces for the different groups
A-B following CA, **C-D** saline at 120 min, **E-F** ALM at 120 min, and **G-H** four controls that failed to achieve ROSC. The complete data for animals can be found in Table 6.2.

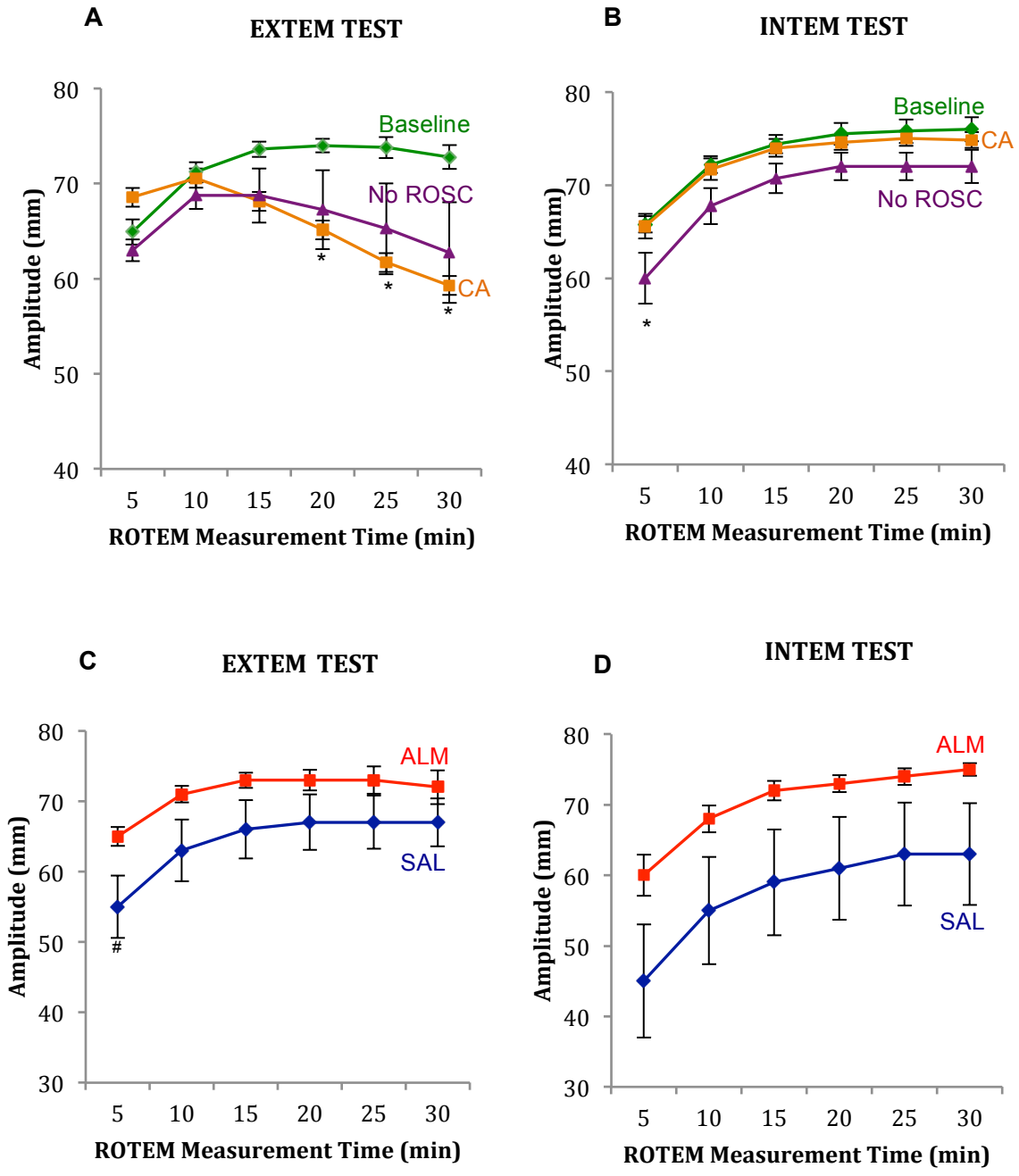


Figure 6.6 EXTEM and INTEM clot amplitudes with time (A5, A10, A15, A20, A25, and A30 min) for the different groups following cardiac arrest

Values represent mean ± SEM. *p<0.05 compared to baseline. #p<0.05 compared to ALM groups.

6.4.5 Standard plasma coagulation test

PTs and aPTTs are shown in Figure 6.7. Cardiac arrest alone did not significantly increase PT and aPTT. In contrast, saline treatment significantly increased both after 120 min ROSC, whereas the ALM group increased only aPTT. Saline controls that did not achieve ROSC increased aPTT but not PT compared with baseline.

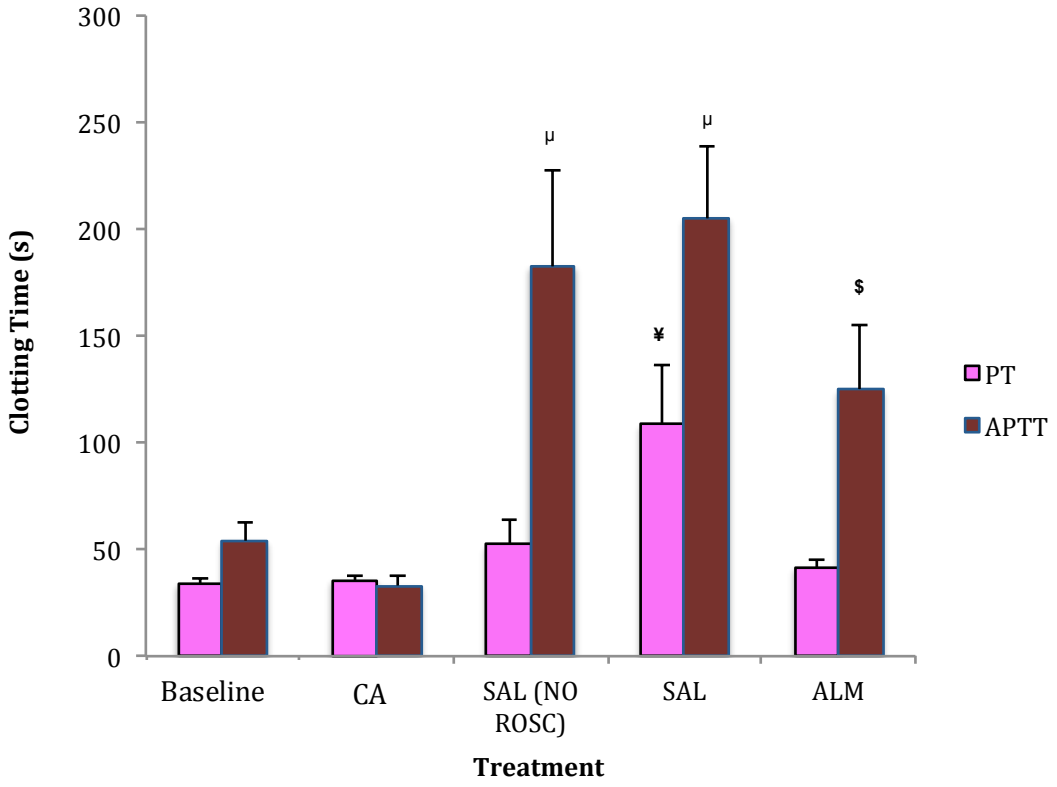


Figure 6.7 Plasma PT and aPTT for the different groups following cardiac arrest
Values are expressed as mean ± SEM. $^{\mu}p<0.05$ compared to baseline and cardiac arrest groups. $^{\$}p<0.05$ compared to cardiac arrest group only. $^{¥}p<0.05$ compared to all other groups except saline controls without ROSC.

6.4.6 Ischaemic neurons in hippocampal CA1 and cortical areas

Eight minute asphyxial hypoxia caused neuronal changes in hippocampal CA1 and cortical region visible as soon as two hour post ROSC. The number of ischaemic neurons in hippocampal and cortical regions found in the saline group were 50 ± 6 and 178 ± 32, respectively, around 12-fold and 10-fold of those found in sham rats (Fig. 6.8). The ALM group had slightly reduced number of ischaemic neurons compared to controls (24% and 34% lower in cortex and hippocampus); however, the presence of these ischaemic neurons was still significantly higher in ALM-treated animals compared to sham in both brain areas.

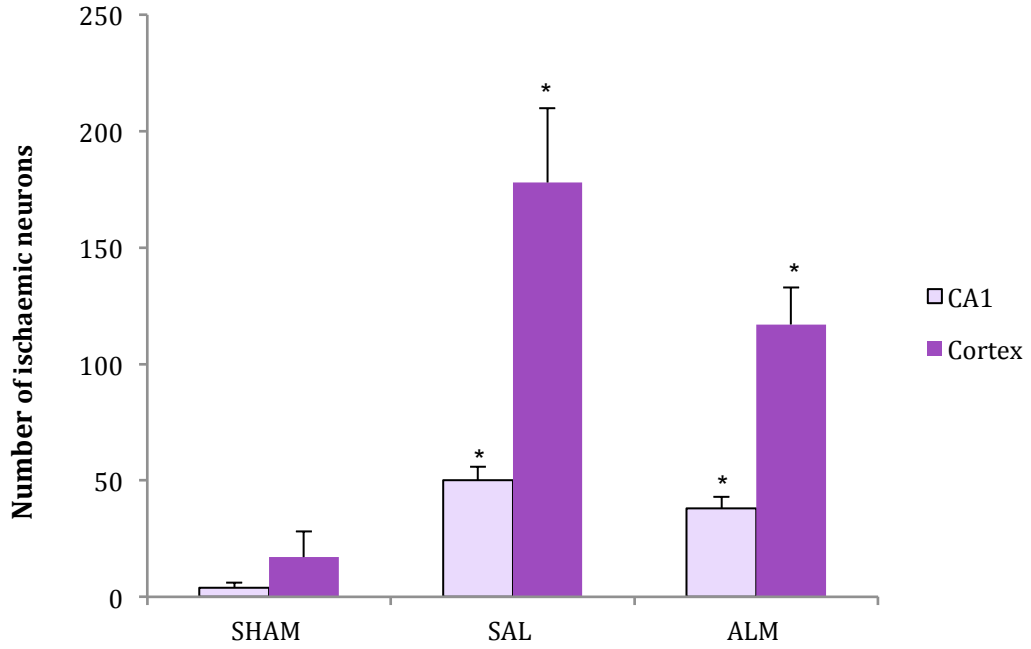


Figure 6.8 The total number of ischaemic neurons found in hippocampal CA1 and cortical region after 8-min asphyxial cardiac arrest in saline and ALM groups
Values are expressed as mean ± SEM. *p<0.05 compared to sham group

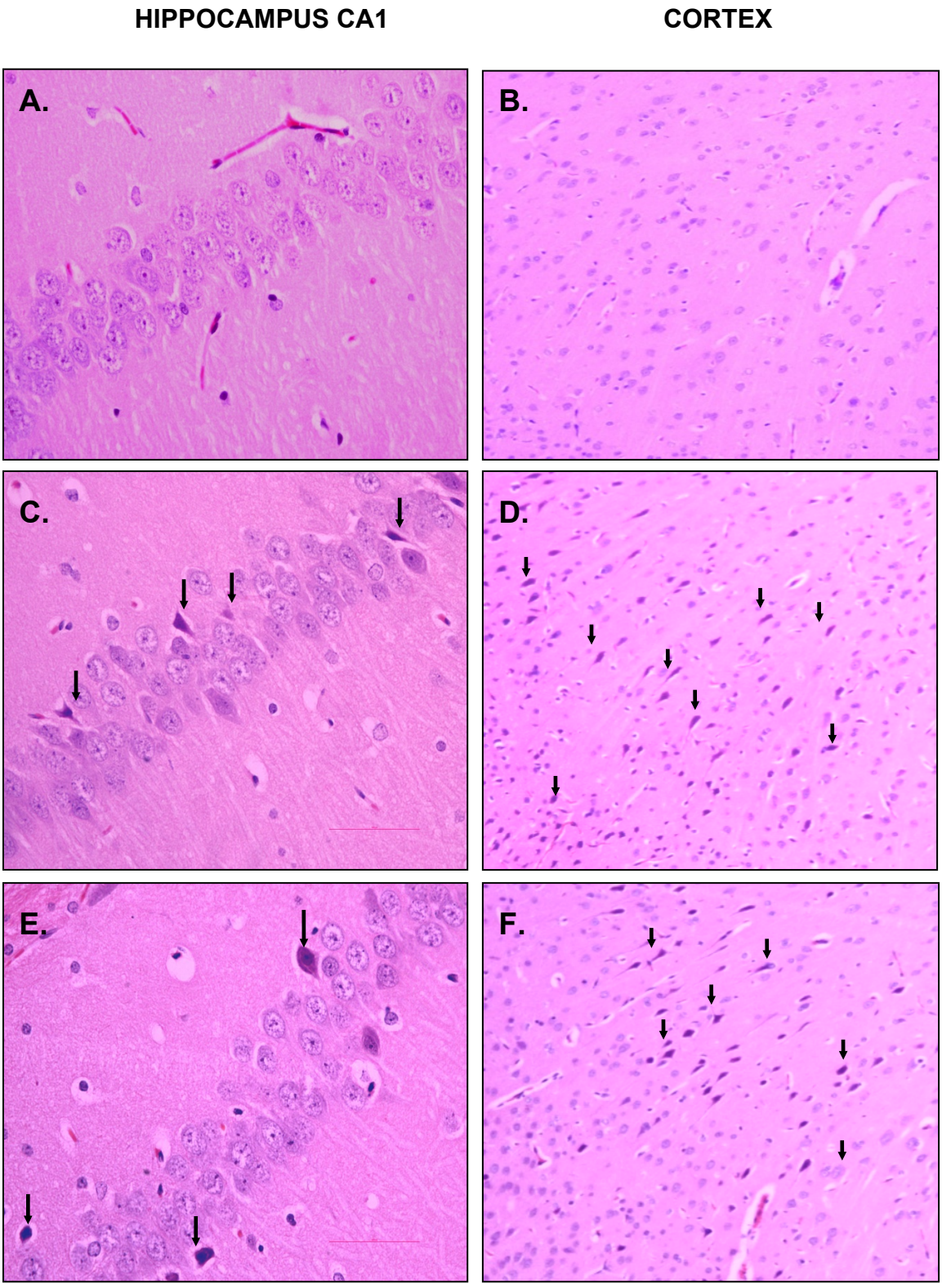


Figure 6.9 Photomicrographs illustrating the number of ischaemic neurons in cortical region of sham rats (A-B) compared to that underwent 8 min asphyxial hypoxia resuscitated with saline (C-D) or ALM (E-F)
Real magnification was 400x for CA1 region and 100x for cortical region. Ischemic neurons (arrows) found scattered in the CA1 area and relatively more diffused in neocortex. No significance difference found between saline and ALM groups. However, the neocortex was slightly less injured in ALM compared to saline group.

6.5 Discussion

This study showed that after intra-arrest hypothermia (~33^{o}C) followed by active rewarming immediately (33-37°C): 1) ALM resuscitation significantly improved ROSC attainment with significantly higher MAPs from 75-120 min compared to saline controls; 2) In contrast to saline controls, ALM prevented a number of coagulation abnormalities associated with cardiac arrest, ROSC and resuscitation, except for INTEM CT; and 3) ALM did not significantly reduce the early neurological changes in neocortex and hippocampus compared to saline controls as assessed by routine histological methods. Thus, there appeared to be no deleterious effect of actively rewarming the animal after hypothermia on ALM's ability to resuscitate MAP or its effect to prevent coagulopathy. These findings and possible clinical significance will now be discussed.

6.5.1 Actively rewarming after intra-arrest hypothermia had no apparent deleterious effect on ALM's ability for cardiac rescue and haemodynamic stabilisation

Following chest compressions after hypothermic asphyxial hypoxia, all ALM-treated rats achieved ROSC when MAP was 36 mmHg, HR was 56 bpm, and rectal temperature was 33^{o}C (Fig. 6.4A-C). In contrast, 33% of the saline controls failed to achieve ROSC from refractory VF, and those rats that did achieve ROSC did so at significantly higher MAP (55 mmHg) and HR (114 bpm) than the ALM group (Fig. 6.4). That 100% of the ALM rats achieved ROSC at such a profound hypotensive state (without inotropes, vasopressors, or fluids) is consistent with data in Chapter 5 and previous published trauma studies. In 2011, Letson and Dobson showed that 0.3 ml 7.5% NaCl ALM, representing <3% total blood shed, rescued MAP into the hypotensive range following severe haemorrhagic shock in rats (Letson & Dobson, 2011a). These results were translated into the porcine model following 75% blood loss and 90-min shock (Granfeldt*, et al.*, 2012). However, the mechanisms for ALM cardiac rescue following asphyxia-hypoxia are not known, and further studies are required to assess the cardiovascular function. What is indicated from the present studies (see Chapter 5), and those from other trauma states (Granfeldt*, et al.*, 2014), is that the ALM's effect appears different from and superior to adenosine (A), lidocaine (L) and magnesium (M) alone.

Although each individual component has cardioprotective properties, the results from Chapter 5 indicate it is the ALM combination that is the key to improved outcomes. For

example, the cardioprotective properties of adenosine against ischaemia-reperfusion injury are well known and linked to: 1) pre-and post-conditioning pathways (Kin*, et al.*, 2005; Vinten-Johansen*, et al.*, 2007); 2) lowering oxygen demand (Shryock & Belardinelli, 1997); 3) ATP preservation from improved mitochondrial protection (Vuorinen*, et al.*, 1995); and 4) increased myocardial glycolysis (Finegan*, et al.*, 1993). In addition, adenosine is a potent anti-inflammatory, prevents platelets from sticking and is a known inhibitor of tissue factor involved in the coagulation pathway (Linden & Cekic, 2012; Vinten-Johansen*, et al.*, 1999). Similarly, a lidocaine bolus has been shown to decrease dP/dt_{max} and lower oxygen demand in rabbits *in* vivo (Lessa & Tibiriçá, 2005). Lidocaine is also an anti-arrhythmic (Wesley, et al., 1991), has free radical scavenging abilities (Lenfant, et al., 2004) and protects against blood clotting (Tobias*, et al.*, 1996). $MgSO_4$ also shares many of these cardioprotective properties, it improves intracellular calcium handling (Ataka*, et al.*, 1993; Iseri & French, 1984), is an anti-arrhythmic (Kiziltepe*, et al.*, 2003), and reduces systemic vascular resistance, arterial pressures and heart rate while maintaining cardiac output (Chakraborti*, et al.*, 2002). Despite these individual properties, each drug does not improve survival or haemodynamics and does not prevent or correct coagulopathy following asphyxial hypoxia (this thesis) or haemorrhagic shock (Letson & Dobson, 2011a; Letson*, et al.*, 2012).

6.5.2 Coagulopathy during cardiac arrest, non-ROSC states, and 120 min after resuscitation with ALM or saline

This present study showed a number of interesting and potentially clinically relevant coagulopathy changes in rats during and following asphyxial-induced cardiac arrest as assessed from whole blood ROTEM analysis and conventional plasmatic coagulometry. In addition to providing cardiovascular rescue and stabilisation, it is found that ALM mostly prevented the coagulation abnormalities after cardiac arrest and resuscitation compared with saline controls.

6.5.2.1 Asphyxial-induced cardiac arrest elicits a hyperfibrinolytic state

ROTEM analysis showed that all animals immediately after 8 min CA had a significant fall in FIBTEM LI, suggesting a progressive hyperfibrinolysis (Table 6.2.). This fall in FIBTEM LI was associated with a significant reduction in EXTEM clot firmness (16%-19% decrease) and EXTEM LI (16%-30% decrease) (Table 6.2). Hyperfibrinolysis is a common coagulopathy following haemorrhagic shock, trauma and cardiac surgery

(Brohi*, et al.*, 2008) and was recently found before hospital admission in human cardiac arrest studies (Schöchl*, et al.*, 2013; Viersen*, et al.*, 2012).

6.5.2.2 Non-ROSC rats were hyperfibrinolytic but with prolonged INTEM CTs

Hyperfibrinolysis was also apparent in saline controls that failed to attain ROSC within 5 min (low LI in EXTEM, INTEM and FIBTEM). Different from during cardiac arrest, non-ROSC rats had prolonged CTs (INTEM, aPTT) and lower amplitudes (INTEM and EXTEM), suggesting weaker clot strength (Fig. 6.5 and Table 6.3). The significance of prolonged CTs in INTEM (and aPTT), but not EXTEM (or PT), may relate to the different contributions of each clotting pathway to the timing and growth of the clot. It is now recognised *in vivo* that the EXTEM (extrinsic) pathway is primarily involved in the initiation of the clot, whereas the INTEM (intrinsic) pathway may be more involved in clot prolongation, expansion and growth (Ovanesov*, et al.*, 2005). These different contributions are time and spatially separate; the extrinsic pathway relying on tissue factor and occurring close to the activated endothelial surface, and the intrinsic arm of clot formation (INTEM) occurring toward the lumen of the vessel (Ovanesov*, et al.*, 2005). This time and spatial interpretation of INTEM data is gaining increasing support from *in vitro* and computer simulation models (Panteleev*, et al.*, 2006).

6.5.2.3 Coagulopathy in ROSC controls at 120 min and its prevention using ALM

Other key findings from the present ROTEM study were: 1) the presence of an acute hypocoagulopathy with clot retraction in saline controls at 120 min without apparent hyperfibrinolysis; and 2) prevention of these ROTEM abnormalities in the ALM-treated rats (Tables 6.3).

Acute hypocoagulopathy at 120 min after ROSC involved both the extrinsic (EXTEM) and intrinsic (INTEM) pathways, presumably from a deficiency or inhibition of one or more factors in the common pathway, and/or a possible fibrinogen defect. Because plasmatic PT and aPTT measurements were also prolonged (Fig. 6.7), it appears that platelets play a minor role in the “blood thinning” defect. Clot retraction was indicated from 15% to 32% decreases in both EXTEM and INTEM MCF and clot amplitudes (Fig. 6.6) with no changes in the EXTEM/FIBTEM LI 30 to 60 min (Table 6.2).
That LI did not change implies the presence of little or no hyperfibrinolysis (Table 6.3) and is consistent with the study of Katori and colleagues (2005), which showed that a decrease in clot amplitude in humans does not always reflect a hyperfibrinolytic state.

A decrease in clot amplitude without fibrinolysis may involve: 1) lower steady-state levels of coagulation Factor XIII released by platelets and neutrophils/macrophages (Bagoly*, et al.*, 2012), 2) a reduced ratio of tissue plasminogen activator/inhibitor ratio (Bolliger*, et al.*, 2012), 3) increased a2-macroglobulin and complement C1 inhibitor (Nayak*, et al.*, 2010), and/or 4) an increase in thrombin-activatable fibrinolysis inhibitor (TAFI) (Katori*, et al.*, 2005). Further studies are required to test these possible mechanisms.

In contrast to saline controls at 120 min, resuscitation with ALM fully prevented: 1) EXTEM hypocoagulopathy (CT, PT); 2) abnormal clot formation (CFT, α angle, MCF, MCE); and 3) clot retraction (Table 6.2, Table 6.3, Fig 6.5). Interestingly, ALM did not prevent prolongation of INTEM CT, and this was also confirmed from high aPTT (Fig. 6.7). However, ALM did partially reduce INTEM CFT increase by ~50% (ALM 56 ± 13 sec, saline 110 ± 4 sec, baseline 32 ± 2.5 sec), but the fall did not reach statistical significance between ALM and baseline or ALM and the saline group because of high standard errors. The higher INTEM variability was not due to heparin contamination, as the rats were not heparinised. It does appear that the small remaining defect in the intrinsic pathway (INTEM) at 120 min after ALM treatment occurs without any measurable defect on clot amplitude (A5-30) or maximal clot elasticity (MCE), as these were comparable to baseline values (Tables 6.2 and 6.3).

Thus, this data appears to support the temporal and spatial separation of the extrinsic and intrinsic clotting pathways, with the intrinsic pathway (INTEM) acting to amplify (but not to initiate) the growth phase of the clot leading to the physical expansion of the fibrin clot (Ovanesov*, et al.*, 2005). In 2012, Letson and colleagues showed that ALM treatment led to a full correction of plasmatic PT and aPTT after ~1 ml/kg 7.5% NaCl ALM resuscitation at 60 min following severe haemorrhagic shock in rats (Letson*, et al.*, 2012).The ability of ALM to correct or maintain clot strength (amplitudes) may be significant since point-of-care low clot strength is an independent predictor of poor prognosis and death in cardiac arrest patients (Kim*, et al.*, 2013).

Table 6.3. Summary of the major coagulation changes over 2 hours of sustained ROSC in the rat model of 8 min asphyxial hypoxia with mild intra-cardiac arrest cooling and rewarming

Group	n	Condition	Observation (Relative to Baseline)	Interpretation
Cardiac Arrest	7	8 min asphyxia (33-34°C)	• No change in clotting times (EXTEM and INTEM) • ↓ Clot firmness (EXTEM) • ↓ EXTEM and FIBTEM Lysis Index	• Time to *initiate* and *elongate* the clot unchanged (EXTEM and INTEM CT) • ↑ Hyperfibrinolysis
SAL	4	Failed to achieve ROSC	• No change in EXTEM, PT clotting times • ↑ Clotting times (INTEM, aPTT) • ↓ Clot firmness (INTEM) • ↓ EXTEM and FIBTEM Lysis Index	• ↑ INTEM CT but not EXTEM (and PT) may indicate prolonged clot *elongation* times with no change in time to *initiate* clot • ↑ Hyperfibrinolysis
SAL	8	After 2 hours of sustained ROSC	• ↑ Clotting times (EXTEM, PT and INTEM, aPTT) • ↑ Clot formation time and angle • ↓ Clot firmness (EXTEM and INTEM) • ↓ Elasticity (40% reduction)	• ↑ times to *initiate* clot (EXTEM) • ↑ times to *elongate* clot (INTEM) • Acute hypocoagulopathy • Clot retraction • No apparent hyperfibrinolysis
ALM	10	After 2 hours of sustained ROSC	• No change in EXTEM clotting time (and PT), formation time, angle, max clot firmness. • ↑ INTEM clot time (aPTT) and formation time • No change clot firmness (EXTEM and INTEM) • No change in elasticity	• Time to *initiate* clot unchanged (EXTEM, PT) • Clot *elongation* time protracted (INTEM, aPTT) • Increase in clot formation time reduced (50% lower than saline controls) • Clot retraction prevented (EXTEM/INTEM) • No apparent hyperfibrinolysis

See experimental protocols and Fig 6.2 for ROTEM definitions

6.5.3 Histopathology changes following cardiac arrest were not significantly reduced with ALM at 120 min of ROSC

Two hours following asphyxial cardiac arrest pyknotic neurons with shrunken eosinophilic cytoplasm were observed in hippocampal CA1 and cortical regions indicating acute ischaemic changes (Figs. 6.8 and 6.9). Furthermore, the present study found the number of ischaemic neurons in control rats was significantly higher (up to 12-fold) compared to sham animals in both observed areas. Evidence of acute ischaemic change one hour post-global ischaemia has previously been demonstrated in a Kawai*, et al.* (1992) study in rats showing the presence of compartmentalised vacuoles and scattered dark and pyknotic neurons in nucleus reticularis thalami (NRT), CA1 sector of hippocampus, cerebral cortex, striatum and substantia nigra.

While ALM treatment reduced the number of ischaemic neurones, and therefore ischaemic damage, in both hippocampal and cortical areas by up to 34%, these data were not significantly different from saline controls (Figs. 6.8 and 6.9). This lack of significance may be due to the low numbers of rats studied in each group or because the two hour post resuscitation time was too early to show a complete neuronal injury profile. Lipinski et al. (1999), and others, have shown that secondary brain injury in rats does not fully develop until 24 hours following asphyxial cardiac arrest (Katz*, et al.*, 1995; Radovsky*, et al.*, 1997). Therefore, future studies are required to increase animal numbers in each group and extend the time of analysis to 24 hours to assess the neurological effects of AL or ALM following asphyxial-induced cardiac arrest.

Previously, Granfeldt and colleagues (2013) also reported no significant difference in neurological scores or neurohistopathological changes between control and AL groups after 24 hours post resuscitation from VF-induced cardiac arrest in swine; and this occurred regardless of improved myocardial function, haemodynamic, and inflammatory reaction in AL group (Granfeldt*, et al.*, 2013). They argued that the ischaemic insult (7-min induced VF) was insufficient to instigate significant histological damage to both cortex and hippocampus areas, thus, further studies are required to examine the potentially neuroprotective properties of AL or ALM in the pig model with increased ischaemia duration and severity (Granfeldt*, et al.*, 2013).

6.5.4 Clinical implications

Translation of any drug therapy from preclinical to clinical studies is fraught with complexity. Nevertheless, ALM therapy may help rescue and stabilise the heart and prevent development of coagulopathy in out-of-hospital asphyxial hypoxia victims and help to restore whole body homeostasis. The ALM therapy may find clinical utility in near-drowning incidents or treating the sudden infant death syndrome or hanging victims. The ability of ALM to maintain clot strength (amplitudes) at point-of-care may be significant as early coagulation abnormalities can progress to DIC, organ failure, and death (Kim*, et al.*, 2013). To date, there has been little success in preventing or correcting coagulopathy with improved outcomes in cardiac arrest patients (Skrifvars & Pettilä, 2013).

6.5.5 Limitation of the study

A potential limitation of the present study is that the haemodynamics and coagulopathy as well as neurological changes were assessed over a two hour post ROSC period. This might be too short to confirm haemodynamic stability and hypocoagulopathy prevention as they may change after 4 to 48 hours following ROSC (Adrie*, et al.*, 2005; Laurent*, et al.*, 2002). In addition, the two hour post-ROSC period may be insufficient to allow the full extent of neurological damage due to delayed nature of neuronal death (Ozawa*, et al.*, 1999). Another limitation is that the application of intra-arrest hypothermia and active rewarming may confound the interpretation of ALM post-resuscitation effects since active rewarming may also affect haemodynamic, neurological and coagulation functions (Scaravilli*, et al.*, 2012). Lastly, immersion fixation was applied to prepare the brain sample for histopathology observation. Immersion fixation may produce artefacts on the brain sections, however, the fixation method is still found useful to detect neurological damage in rat brains (Kasukurthi*, et al.*, 2009).

6.6 Conclusion

ALM i.v injection during hypothermic resuscitation improved survival and significantly improved haemodynamics during rewarming after asphyxial-induced cardiac arrest. Hyperfibrinolysis occurred at 8-min cardiac arrest. At 120 min after ROSC, saline controls showed prolonged CTs, reduced MCF, and decreased clot amplitudes with no hyperfibrinolysis. These abnormalities were fully prevented by ALM with exception

of INTEM CT at 120 min after ROSC. In addition to acute coagulopathy, the presence of ischaemic neurons was apparent after two hours post-ROSC. ALM treatment slightly reduced the number of ischaemic neurons, in both hippocampal and cortical areas, but the results were not significantly different from saline controls.

CHAPTER 7. EFFECT OF ALM AND EPINEPHRINE ON HAEMODYNAMIC AND CARDIAC RHYTHM STABILITY FOLLOWING 8 MIN ASPHYXIAL HYPOXIA IN RATS

7.1 Introduction

Having established that small-volume ALM improves early survival and prevents coagulopathy following 8 min asphyxial hypoxia, this chapter will compare these data with standard-of-care epinephrine under identical cold-arrest and rewarming conditions used in Chapter 6.

As mentioned in Chapter 1, epinephrine is a mixed adrenergic agonist affecting α- and β-receptors and is the preferred standard-of-care treatment for out-of-hospital cardiac arrest victims (Callaway, 2013). The rationale for its use as the first-line resuscitation drug is to elicit peripheral vasoconstriction through α-adrenergic receptor stimulation and redirect blood flow to central organs (Callaway, 2012; Michael*, et al.*, 1984), resulting in higher aortic pressures and higher driving pressures for coronary and cerebral perfusions (Paradis*, et al.*, 1990; Reynolds*, et al.*, 2010). However, through β-adrenergic activation, epinephrine increases cardiac workload and oxygen consumption, which may increase the severity of post-resuscitation myocardial dysfunction (Angelos*, et al.*, 2008; Berg*, et al.*, 1994; Lindner*, et al.*, 1991; Tang*, et al.*, 1995).

Two recent clinical trials confirmed that epinephrine in prehospital cardiac arrest does increase the likelihood of achieving ROSC compared to placebo, but failed to demonstrate improved survival to hospital discharge (Hagihara*, et al.*, 2012; Jacobs*, et al.*, 2011a). The present study is designed to compare ALM's effects with standard-of-care treatment epinephrine following asphyxial hypoxia with intra-arrest hypothermia and post-ROSC rewarming. Whole blood ROTEM and plasma PT/aPTT analyses were also performed after two hours of ROSC.

7.2 Aims

To compare the effects of ALM and epinephrine (0.01 mg/kg) on ROSC, post-ROSC haemodynamics, ECG and coagulation status following asphyxial-induced cardiac

arrest in rats following 8 min intra-arrest hypothermia (~33^{o}C) and 120 min active rewarming (33-37°C).

7.3 Experimental Protocols

7.3.1 Experimental design

Animal housing, feeding and water regimes, ethics approvals, methods of anaesthesia and surgical protocols are described in Chapter 2.

Non-heparinised rats (350-450g) were randomly assigned to one of two groups: 1) ALM (n=12) and 2) epinephrine (EPI, n=12). A separate group of 12 healthy animals were used for baseline coagulation analysis. These animals underwent surgical procedures but no temperature manipulation or asphyxiation. The ALM group in this chapter was a separate group from Chapter 6. However, saline controls presented in Table 7.3 for coagulopathy were those used in Chapter 6, and obtained under identical conditions as those described in the present chapter.

The 0.5 ml ALM dose had identical composition to previous chapters as shown in Fig 7.1. The epinephrine IV bolus dose was 0.01 mg/kg body weight. The 0.5 ml epinephrine bolus dose of 0.01 mg/kg was chosen based on preliminary experiments showing that higher doses (0.04 or 0.1 mg/kg) resulted in major adverse effects such as uncontrolled haemorrhage from the nose and mortality within 20 min of achieving ROSC (data not presented).

The hypothermic cardiac arrest and rewarming resuscitation protocols have been described in detail in Chapter 6 (Djabir*, et al.*, 2013). Surface cooling (ice packs and ethanol spray) was applied during asphyxiation to target 33^{o}C at the time of resuscitation and rewarming at a rate of ~0.02-0.06^{o} C/min with heating lamp started as soon as ROSC was achieved (see Figs 7.1 and 7.2). Haemodynamic measurements have been previously described, with the addition of rate-pressure product (RPP) as an index of myocardial oxygen consumption (White, 1999), calculated from the following equation: RPP = Peak Systolic Pressure (SAP) x Heart Rate (HR).

Coagulation studies were performed using plasmatic PT/aPTT and whole blood ROTEM analyses assessed at baseline and 120 min as described in Chapters 2 and 6.

Definitions: ROSC was defined as a SAP >20 mmHg. Cardiac arrest was defined as a MAP <10 mmHg (Fig 7.1). Survival was defined as those animals that achieved and maintained ROSC up to two hours. Episodes and duration of arrhythmias were recorded using lead II ECG and identified using the Lambeth convention, as previously outlined in Chapter 3 and Canyon and Dobson (2004). Venticular fibrillation (VF) was defined as QRS deflections that could not be easily distinguished and HR that could not be measured, and ventricular tachycardia (VT) was defined as four or more consecutive ventricular premature beats (premature QRS complexes).

7.3.2 Statistical Analysis

Statistical analysis was performed using SPSS statistical package (PASW statistic 18). All values are expressed as mean ± SEM. Survival and arrhythmia incidence were analysed with *Chi-Square* test. Data distribution was tested with *Kolmogorov-Smirnov* for normality. Normal distributed data (coagulation parameters, PT and aPTT data) was analysed using one-way ANOVA followed by *Tukey*'s post-hoc test, while haemodynamic comparisons between ALM and EPI groups were analysed with independent T-test. Data that was not normally distributed (ROTEM CFT, α angle, LI30-60) was analysed using *Kruskal-Wallis* test and *Mann-Whitney U* test. The pair-wise comparisons were only applied when *Kruskal-Wallis* test was significant. Statistical significance is defined as a $p<0.05$.

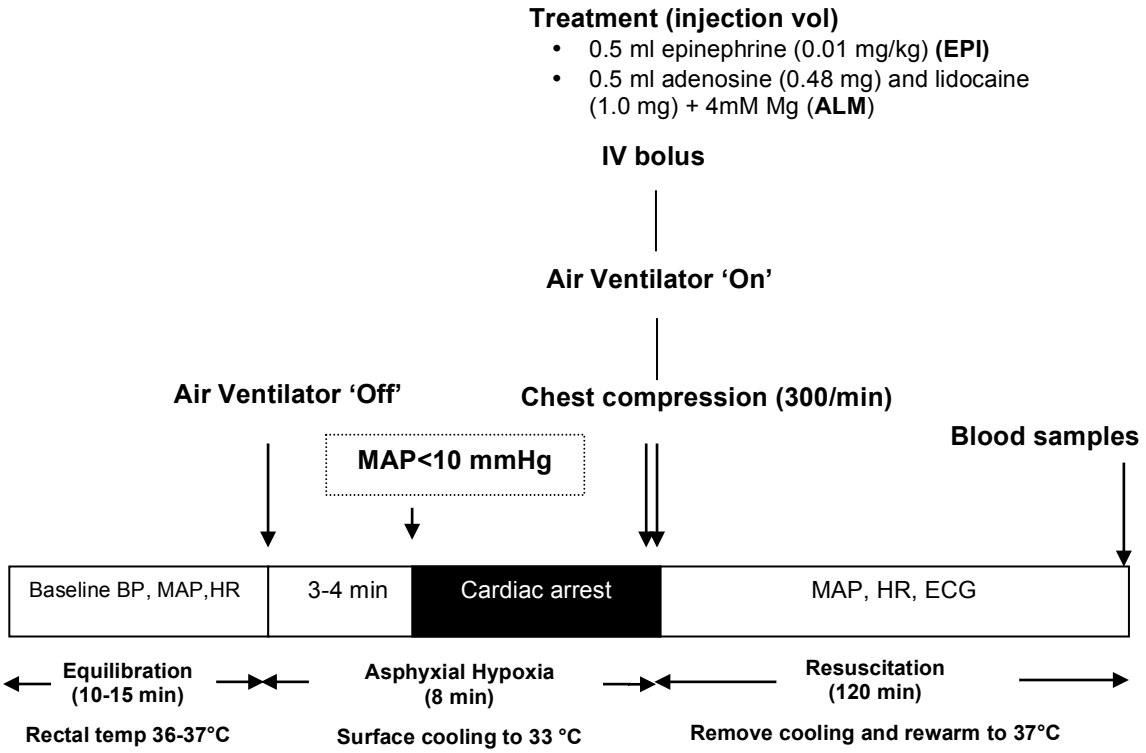


Figure 7.1 A schematic of the experimental protocol in 8 min asphyxial hypoxia rat model
Surface cooling (ice pack and ethanol spray) was applied during asphyxia to target 33^{o}C during resuscitation. As soon as ROSC was achieved, rewarming with heating lamp was applied immediately. Blood samples were withdrawn at the end of experiment (120 min post ROSC).

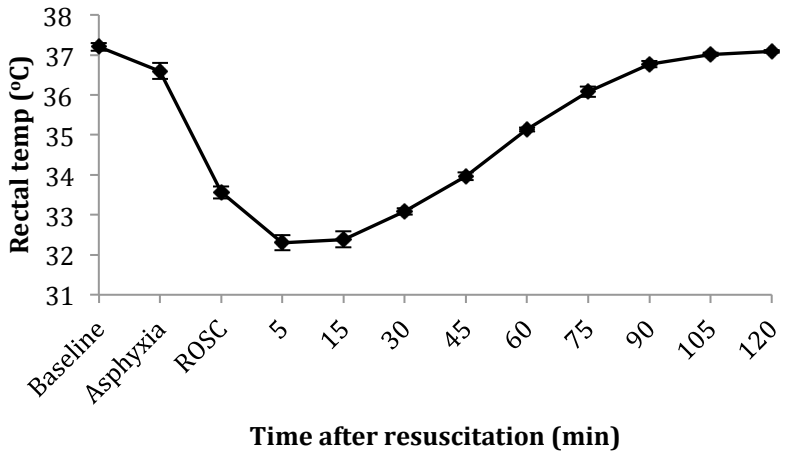


Figure 7.2 Average rectal temperature (^{o}C) during baseline, initiation of asphyxia, onset of ROSC and times following resuscitation (n=24)

7.4 Results

7.4.1 Baseline and cardiac arrest data among treatment groups

Baseline haemodynamic data and cardiac arrest time are presented in Table 7.1, and were not significantly different between treatment groups. Baseline MAPs were 124 ± 3.3 and 124 ± 2.6 mmHg for ALM and epinephrine groups, respectively, whereas the baseline heart rates were 371 ± 7.2 and 375 ± 6.8 bpm, respectively. Baseline temperature in all groups was 37^{o}C. Times to cardiac arrest were 215 ± 13.0 and 222 ± 13.4 sec, while cardiac arrest durations were 259 ± 4.8 and 258 ± 5.6 sec respectively for ALM and EPI groups.

Table 7.1 Baseline haemodynamics and cardiac arrest time among treatment groups

Group	n	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (bpm)	Temp (^{o}C)	Time to CA (s)	Duration of CA (s)
ALM	12	132 ± 3.6	120 ± 3.2	124 ± 3.3	371 ± 7.2	37.0 ± 0.1	215 ± 13.0	259 ± 4.8
EPI	12	139 ± 3.5	116 ± 2.3	124 ± 2.6	375 ± 6.8	37.2 ± 0.2	222 ± 13.4	258 ± 5.6

Values represent mean ± SEM. SAP = arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, HR = heart rate, Temp = rectal temperature, Time to CA = time from the initiation of asphyxia to cardiac arrest, Duration of CA = duration from cardiac arrest to the onset of chest compressions. No significant difference found between groups.

7.4.2 Haemodynamics during chest compressions, ROSC achievement, and the incidence of arrhythmias following ROSC with ALM or EPI

Following 60 sec of chest compressions all rats in ALM group achieved ROSC, while 3 out of 12 animals (25%) in EPI group did not attain ROSC due to protracted VF. The SAP and DAP during chest compressions in ALM group were 49 ± 3.3 and 39 ± 2.8 mmHg, respectively, and significantly lower than those of EPI group (79 ± 6.4 and 64 ± 6.1 mmHg) (Table 7.2). Three ALM-treated rats experienced non-sustainable VTs but no ALM rats converted to VF. The number of EPI-treated animals with arrhythmias (VT/VF) were two times higher than those of ALM group (6 and 3 animals, respectively), however, this difference was not statistically significant (Table.7.2).

Table 7.2 Arterial pressures during chest compression, resuscitation outcome and number of animal with ventricular arrhythmias (VT/VF) following different treatments

		Chest compression		Resuscitation outcome		Animals with ventricular arrhythmias		
Group	n	SAP (mmHg)	DAP (mmHg)	ROSC (%)	No ROSC (%)	VT n (%)	VF n (%)	VT/VF n (%)
ALM	12	49 ± 3.3	39 ± 2.8	12 (100)	0 (0)	3 (25)	0 (0)	3 (25)
EPI	12	79 ± 6.4*	64 ± 6.1*	9 (75)	3 (25)	3 (25)	3 (25)	6 (50)

Value expressed as mean ± SEM. SAP = systolic arterial pressures, DAP = diastolic arterial pressures, ROSC = return of spontaneous circulation, VT = ventricular tachycardia, VF = ventricular fibrillation, VT/VF = Ventricular tachycardia and/or ventricular fibrillation. *p<0.05 compared to ALM.

7.4.3 Effect of ALM and EPI on arterial pressures following ROSC

Figure 7.3 shows the arterial pressures following resuscitation with the two different treatments. Immediately after ROSC (time 0), rats treated with ALM had SAP of 59 ± 5.0 mmHg, DAP of 29 ± 2.7 mmHg and MAP of 39 ± 3.3 mmHg (Fig. 7.3A-C). In contrast, the arterial pressures of EPI group were significantly higher with SAP of 159 ± 9.4 mmHg, DAP of 114 ± 6.6 mmHg and MAP of 129 ± 7.0 mmHg. These extremely high pressures in the EPI-treated rats were ~three-fold higher than those of ALM-treated rats. The EPI-treated pressures were so high early that they surpassed their baseline values, with one rat having 1.6 fold higher SAP than its basal SAP (data not shown). The arterial pressures in EPI-treated groups then fell abruptly after 5 min; however, these pressures were still significantly higher than those generated by the ALM group (Fig 7.3).

After 30 min ROSC, the arterial pressures in ALM group continually increased (Fig. 7.3A-C). In contrast, in EPI group between 90 and 120 min, the arterial pressures gradually decreased, with more pronounced decreases in the diastolic pressures (Fig. 7.3B). Between 90-120 min, the SAP, MAP and DAP were significantly lower in EPI group compared to the ALM group (Fig. 7.3A-C). At 120 min, ALM group had the SAP of 91 ± 4.0 mmHg, DAP of 75 ± 2.3 mmHg and MAP of 81 ± 2.9 mmHg, which were all significantly higher than those in the EPI group (SAP of 72 ± 3.1 mmHg, DAP of 56 ± 1.6 mmHg and MAP of 62 ± 3.9 mmHg).

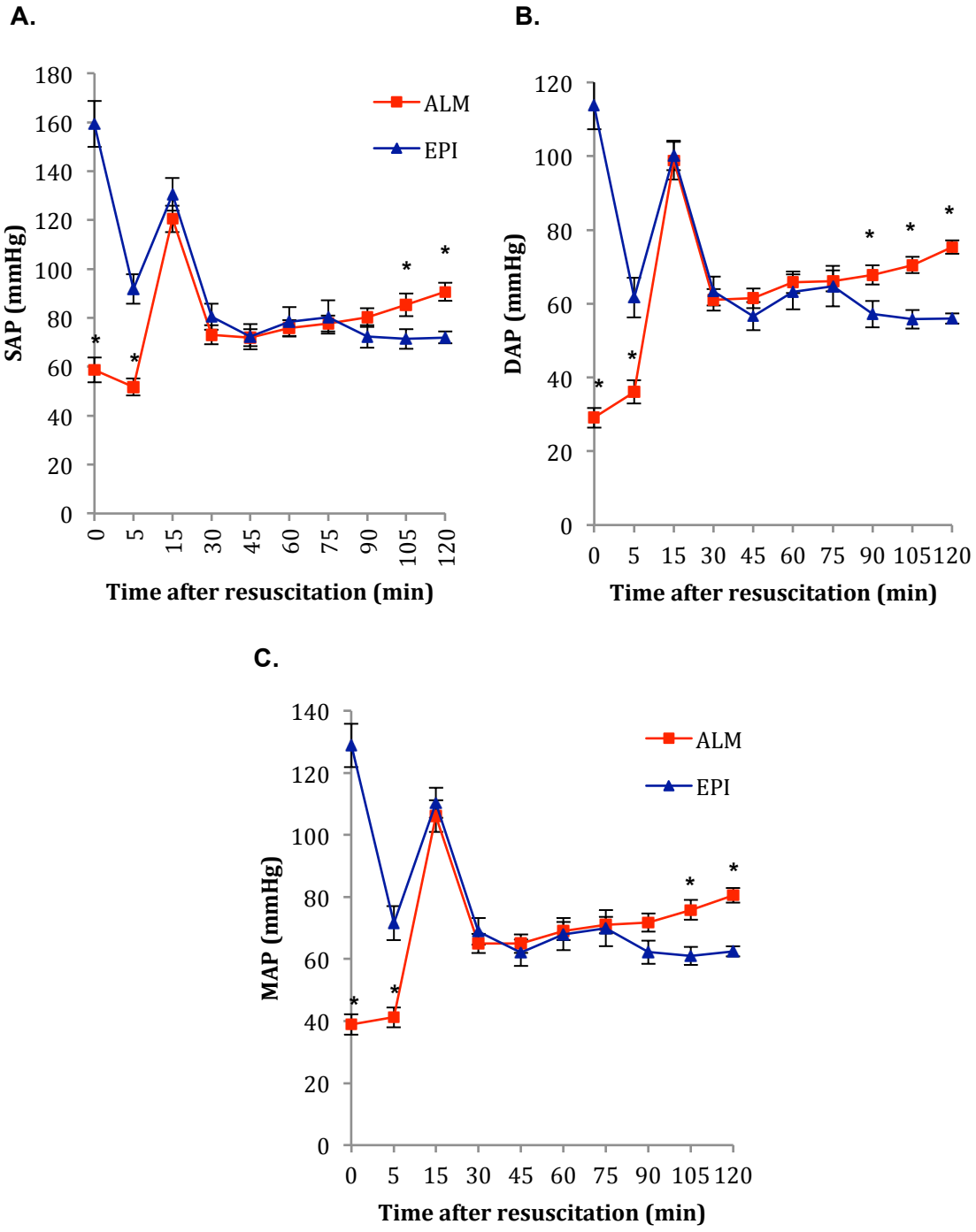


Figure 7.3 A. Systolic arterial pressures (SAP), B. Diastolic arterial pressures (DAP), and C. Mean arterial pressures (MAP) following resuscitation during 2 hour of ROSC
Values are expressed as mean ± SEM. *p<0.05 ALM compared to EPI group

7.4.4 Effect of ALM and EPI on heart rate and rate-pressure product following ROSC

Immediately post-ROSC, heart rate of the EPI group was significantly higher than the ALM-treated rats (151 ± 13.6 bpm vs 59 ± 5.5 bpm) (Fig. 7.4A). At 5 min, the heart rate for both groups increased but the ALM group remained significantly lower than the EPI group (176 ± 8.8 bpm vs. 227 ± 12.7 bpm). After 15 min, the heart rate of the ALM group increased and was not significantly different from the EPI group. At 120 min, ALM and EPI groups had augmented heart rates to 334 ± 7.7 bpm and 318 ± 7.0 bpm, respectively, which are ~90% of baseline (~370 bpm) (Table 7.1).

Immediately post-ROSC, the rate-pressure product (RPP) for the EPI-treated group went from zero during arrest to 24,119 ± 3,037 mmHg.bpm, and was 5.6 times higher ($P<0.05$) than in rats treated with ALM (4,279 ± 917 mmHg.bpm) (Fig. 7.4B). At 5 min, RPP in the EPI group fell by ~20% then rapidly increased again to a maximum of 34,692 ± 2,778 mmHg.bpm at 15 min. This transient drop in RPP did not occur in the ALM group. In the ALM group, a significantly lower RPP was maintained in the first 5 min after ROSC and then between 5 and 15 min, RPP increased at a similar rate to the EPI group, and the values were not significantly different (Fig. 7.4B). In both groups, at 30 min RPP fell by around 30% then increased between ~25,000 to ~26,000 mmHg.bpm at 75 min. After 75 min, RPP fell to ~23,000 mmHg.bpm, whereas ALM RPP continued to increase, and was significantly higher than the EPI group at 105 and 120 min.

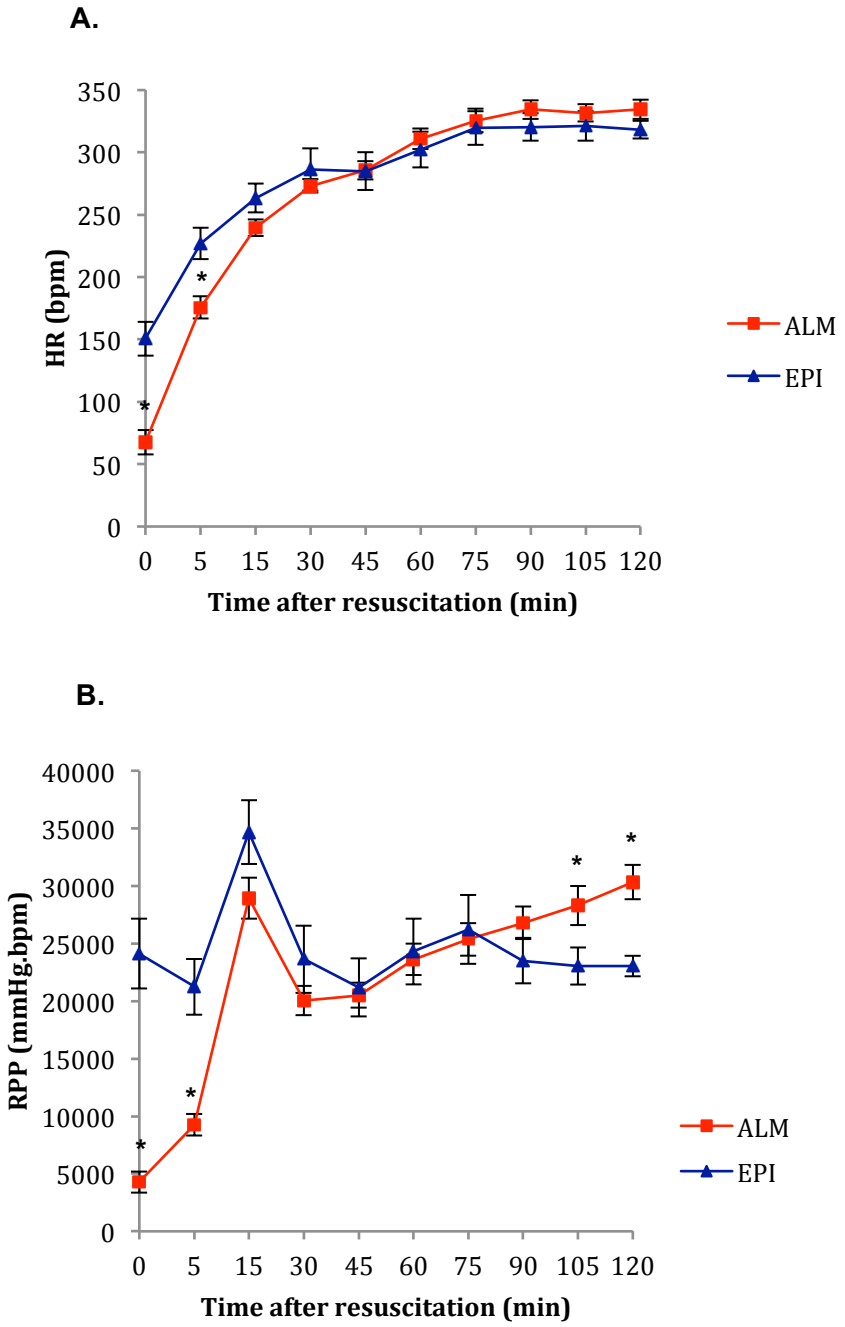


Figure 7.4 A. Heart rate (HR) and B. Rate pressure products (RPP) following resuscitation during 2 hour of ROSC
Values are expressed as mean ± SEM. *p<0.05 ALM compared to EPI group

7.4.5 PT and aPTT analysis

Values for PT and aPTT times for ALM and EPI groups are found in Fig. 7.5. Baseline plasma PT and aPTT values were 36.2 ± 2.8 sec and 43.7 ± 3.9 sec, respectively. At 120 min following ROSC, PT values in the ALM- or EPI-treated rats were not significantly different from baseline and ranged between 37.4 sec and 37.8 sec. In contrast, aPTT increased by over two-fold in both groups compared to baseline (Fig. 7.5). The aPTT for the ALM group at 120 min was 108.5 ± 27.8 sec, and the aPTT for the EPI group was 103.9 ± 24.4 sec (only ROSC survivors). In the EPI-treated rats that did not attain ROSC, the PT was 1.4 times baseline (49.3 ± 9.3 sec) and the aPTT was three times baseline (129.7 ± 51.1 sec). However, the extended aPTT values were not considered significantly different to the baseline due to high variability (wide SEM) in the treatment groups (Fig. 7.5).

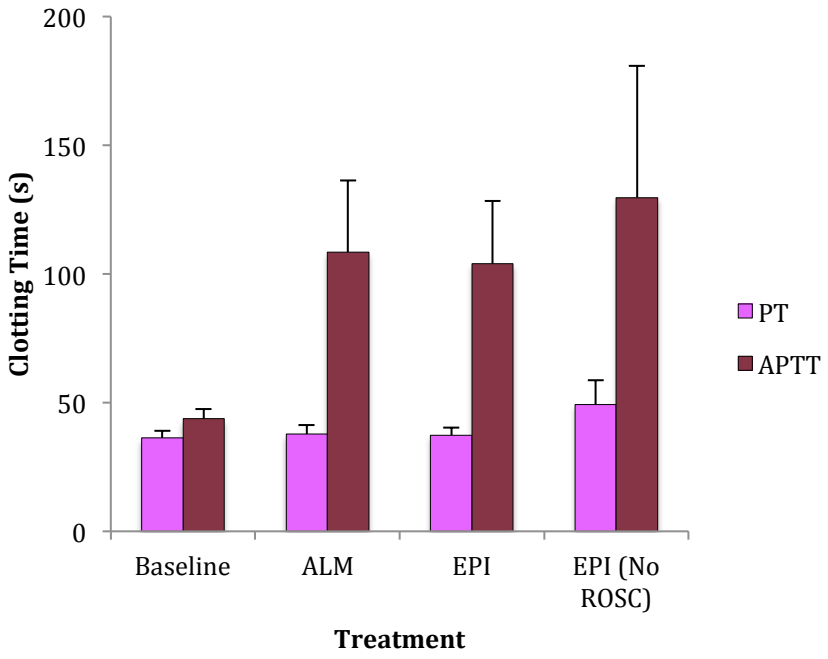


Figure 7.5 Plasma prothrombin time (PT) and activated partial thromboplastin time (aPTT) for different groups following cardiac arrest
Values are expressed as mean ± SEM.

Table 7.3 Effect of cardiac arrest and small-volume ALM and epinephrine (0.01 mg/kg) on ROTEM coagulation parameters following 8 min of asphyxial cardiac arrest

Groups	Test	Coagulation parameters													
		CT (s)	CFT (s)	α (º)	MCF (mm)	A5 (mm)	A10 (mm)	A15 (mm)	A20 (mm)	A25 (mm)	A30 (mm)	LI30 (%)	LI45 (%)	LI60 (%)	MCE
Base-line	EXTEM	39 ± 2.4	33 ± 1.7	83 ± 0.4	75 ± 0.7	65 ± 1.1	71 ± 1.0	73 ± 0.7	74 ± 0.6	73 ± 1.0	72 ± 1.1	98 ± 0.9	91 ± 2.5	87 ± 3.0	294 ± 10.0
	INTEM	119 ±11.6	34 ± 3.0	83 ± 0.6	76 ± 1.0	65 ± 0.9	72 ± 0.8	74 ± 0.9	75 ± 1.0	76 ± 1.1	76 ± 1.1	100 ± 0.3	99 ± 0.4	98 ± 0.6	322 ± 14.7
n= 12	FIBTEM	37 ± 1.7	**NA**	79 ± 0.9	15 ± 0.5	15 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.5	100 ± 0.2	100 ± 0.0	100 ± 0.0	18 ± 0.8
With ROSC (t=120 min)															
Saline#	EXTEM	71 ± 12.2[¥]	68 ± 20.1	77 ± 3.4	68 ± 3.4	55 ±4.4[∞]	63 ± 4.4	66 ± 4.2	67 ± 3.9	67 ± 3.8	67 ± 3.4	98 ± 1.0	91 ± 3.5	87 ± 4.4	231 ± 22.4
n=8	INTEM	212 ±50.9[∞]	110 ± 33.8[£]	66 ±8.7[£]	64 ± 6.7[✗]	45 ±8.0[£]	55 ± 7.6[✗]	59 ± 7.5[✗]	61 ± 7.3[✗]	63 ± 7.3[✗]	63 ± 7.2[✗]	100 ± 0.0	100 ± 0.2	99 ± 0.6	222 ± 37.5[✗]
	FIBTEM	91 ± 29.9	**NA**	77 ± 1.1	14 ± 1.9	12 ± 1.7	12 ± 1.8	13 ± 1.9	13 ± 1.9	14 ± 1.9	14 ± 1.8	100 ± 0.4	100 ± 0.4	99 ± 0.6	17 ± 2.7
ALM	EXTEM	42 ± 4.1	39 ± 4.3	83 ± 0.9	74 ± 1.1	64 ± 1.6	70 ± 1.2	73 ± 1.1	73 ± 1.2	73 ± 1.4	73 ± 1.7	98 ± 1.2	93 ± 2.1	89 ± 2.6	296 ± 19.2
n= 12	INTEM	177 ±38.1	50 ± 11.5	80 ± 2.0	76 ± 0.9	62 ± 2.7	70 ± 1.8	73 ± 1.4	74 ± 1.2	75 ± 1.1	76 ± 1.0	100 ± 0.0	100 ± 0.1	99 ± 0.4	311 ± 14.7
	FIBTEM	45 ± 4.8	**NA**	75 ± 2.0	14 ± 0.8	13 ± 0.7	14 ± 0.8	14 ± 0.7	14 ± 0.7	14 ± 0.8	15 ± 0.8	100 ± 0.1	100 ± 0.0	100 ± 0.0	17 ± 1.1
EPI	EXTEM	49 ± 7.8	41 ± 7.1	82 ± 1.4	74 ± 1.1	64 ± 2.3	70 ± 1.7	73 ± 1.3	74 ± 1.2	74 ± 1.2	74 ± 1.3	99 ± 0.7	97 ± 1.3	94 ± 1.5	294 ± 15.8
n=9	INTEM	158 ±15.3	33± 2.6	84 ± 0.6	77 ± 0.9	65 ± 1.7	72 ± 1.3	74 ± 1.1	76 ± 1.0	76 ± 0.9	77 ± 0.9	100 ± 0.0	100 ± 0.3	98 ± 0.6	333 ± 15.6
	FIBTEM	38 ± 11.4	**NA**	75 ± 2.3	15 ± 0.9	15 ± 2.0	14 ± 0.9	15 ± 1.0	15 ± 1.0	15 ± 1.0	15 ± 1.1	100 ± 0.2	100 ± 0.1	100 ± 0.0	17 ± 1.4
Without ROSC (t<5 min)															
Saline#	EXTEM	43 ± 3.2	36 ± 2.6	83 ± 0.6	71 ± 1.4	63 ± 2.0	69 ± 1.7	69 ± 2.1	67 ± 3.0	64 ± 3.5	63 ± 3.5	89 ± 3.4*	77 ± 4.7[✗]	69 ± 7.0*	241 ± 17.4
n=4	INTEM	175 ±28.6	49 ± 8.7	80 ±1.8[¢]	73 ± 1.2	60 ± 2.7	68 ± 1.9[∞]	71 ± 1.6	72 ± 1.5[∞]	72 ± 1.5[∞]	72 ± 1.8	97 ± 1.9	95 ± 3.5	93 ± 5.4	280 ± 16.7
	FIBTEM	42 ± 1.3	**NA**	73 ± 2.8	14 ± 2.1	13 ± 2.4	13 ± 2.1	13 ± 2.1	14 ± 1.9	14 ± 1.9	14 ± 1.9	100 ± 0.0	99 ± 1.5	97 ± 2.1[✗]	16 ± 3.0
EPI	EXTEM	38 ± 1.5	35 ± 2.7	84 ± 0.3	72 ± 2.1	66 ± 0.6	71 ± 0.9	71 ± 2.6	68 ± 4.6	65 ± 4.9	62 ± 4.9	85 ± 4.3*	73 ± 6.0*	58 ± 8.5*	264 ± 26.4
n=3	INTEM	157 ±22.6	36± 2.7	83 ± 0.6	74 ± 3.0	64 ± 0.6	70 ± 1.2	72 ± 2.3	72 ± 3.5	72 ± 4.8	71 ± 6.0	96 ± 4.3	91 ± 8.7	89 ± 10.7	295 ± 40.0
	FIBTEM	36 ± 4.8	**NA**	79 ± 1.8	18 ± 2.4	17 ± 2.6	17 ± 2.7	17 ± 2.5	18 ± 2.4	18 ± 2.4	18 ± 2.5	100 ± 0.0	100 ± 0.0	100 ± 0.0	21 ± 3.8

ROTEM analysis performed 120 min post ROSC following 8 min of asphyxial-induced cardiac arrest. Data also includes saline and epinephrine-treated rats that did not achieve ROSC. Value are expressed as mean ± SEM. CT = Clotting time, CFT = Clot Formation time, α = angle between initiation of clot and clot amplitude, A(5-30) = clot amplitude at 5-30 min, LI30-60 = Lysis index at 30-60 min, MCE = Maximum clot elasticity. **#**Saline data was taken from Chapter 6 study to provide a direct comparison to ALM and EPI treatments. [¥]p<0.05 compared to all other groups except saline without ROSC. [∞]p<0.05 compared to baseline only *p<0.05 compared to baseline and all other groups with ROSC. [✗]p<0.05 compared to baseline, ALM and EPI with ROSC. [£]p<0.05 compared to baseline and EPI with ROSC. [¢]p<0.05 compared to EPI with ROSC only.

7.4.6 ROTEM Coagulation Parameter

The results of ROTEM analysis (EXTEM, INTEM and FIBTEM) performed at baseline and 120 min post-resuscitation are presented in Table 7.3. The EXTEM and INTEM test were performed to assess the contributions of extrinsic and intrinsic pathways to whole blood coagulation, while the FIBTEM test examines the role of fibrinogen in clot polymerization without platelet function.

7.4.6.1 EXTEM analysis

In healthy rats (baseline), the EXTEM clotting time parameters CT and CFT were 39 ± 2.8 sec, 33 ± 1.9 sec; the clot firmness indicators α and MCF were 83 ± 0.4^{o} and 75 ± 0.8 mm; while the lysis parameters LI30, LI45 and LI60 were 98 ± 1.0%, 91 ± 2.7%, and 87 ± 3.2%, respectively.

Saline controls: The effect of saline controls on EXTEM ROTEM analysis was a significant prolongation of CT, prolonged CFT (two-fold increase) and significantly reduced clot amplitude (A5) compared to baseline (see Table 7.3).

ALM and EPI survivors: After 2 hours of ROSC, ALM treatment resulted in the following ROTEM values: EXTEM CT 42 ± 4.1 sec; CFT 39 ± 4.3; MCF 74 ± 1.1 mm; LI30 98 ± 1.2%; LI45 93 ± 2.1% and LI60 of 89 ± 2.6% (Table 7.3). These EXTEM values for ALM-treated animals at 120 min were similar to baseline values in healthy animals.

The EPI-treatment group had similar EXTEM clotting time and firmness (CT, CFT, α, MCF, A5-30) to baseline values with small increases in lysis indices (LI45 = 97 ± 1.3 %, LI60 = 94 ± 1.5%) compared to normal rats (LI45 = 91 ± 2.5%, LI60 = 87 ± 3.0%), but these were not statistically significant.

Non-survivors in EPI group: After 8 min cardiac arrest, those rats that did not attain ROSC (n=3 from the EPI group) showed comparable clot times and clot firmness to baseline values (non-ROSC EXTEM CT = 38 ± 1.5 sec, CFT = 35 ± 2.7 sec, MCF = 72 ± 2.1 mm). However, the clot amplitude was significantly reduced after 25-30 min (A25-30) and this led to significantly lower lysis indices (non-ROSC EXTEM LI 30 = 85 ± 4.3%, LI45 = 73 ± 6.0%, LI60 = 58 ± 8.5%) compared to baseline (Table 7.3).

7.4.6.2 INTEM analysis

The baseline values for INTEM CT, CFT, MCF, LI30, LI45 and LI60 were 119 ± 11.6 sec, 34 ± 3.0 sec, 76 ± 1.0 mm, 100 ± 0.3%, 99 ± 0.4%, 98 ± 0.6%, respectively (Table 7.3).

Saline controls: The effect of saline controls on INTEM ROTEM analysis was a significant prolongation of clotting times (CT and CFT, up to three-fold increase), significantly reduced alpha angle, MCF and clot amplitudes (A5-30) compared to baseline.

ALM and EPI survivors: After two hours of ALM treatment, both INTEM CT and CFT were slightly lengthened (177 ± 38.1 and 50 ± 11.5 sec, respectively). In the EPI-treated group, only INTEM CT (158 ± 15.3 sec), but not CFT (33± 2.6 sec), was slightly prolonged. Apart from the prolonged INTEM clotting times, all the other INTEM parameters (α, MCF, A5-30, LI30-60, MCE) were not significantly different between the ALM, EPI and baseline groups (Table 7.3).

Non-survivors in EPI group**:** Following cardiac arrest, the rats that failed to be rescued in EPI group had somewhat prolonged INTEM CT (157 ± 22.6 sec) but the CFT was unchanged (36 ± 2.7 sec). Clot firmness (α, MCF and A5-30) in these non-survivors was relatively similar to baseline, however, the INTEM lysis indices were fairly reduced with LI45 of 91 ± 8.7% and LI60 of 89 ± 10.7% compared to LI45 of 99 ± 0.4% and LI60 of 98 ± 0.6% in baseline group. Again, this reduction in lysis indices was not statistically significant.

7.4.6.3 FIBTEM analysis

In normal rats, baseline values for FIBTEM CT, α, MCF, A10, LI30-60 were 37 ± 2.7 sec, 79 ± 1.4^{o}, 15 ± 0.7 mm, 15 ± 0.8 mm, 100 ± 0.0%, respectively (Table 7.3).

Saline controls: The effect of saline controls on FIBTEM ROTEM analysis was a prolongation of clotting time (2.5 fold increase) with little change to clot amplitudes compared to baseline.

ALM and EPI survivors: Both ALM and EPI treatment groups showed little or no change in FIBTEM parameters, including CT, α, MCF, A10, LI30, LI45 and LI60. ALM and EPI rats had FIBTEM CT of 45 ± 4.8 and 38 ± 11.4 sec; FIBTEM α of 75 ± 2.0 and

75 ± 2.3°; FIBTEM MCF of 14 ± 0.8 and 15 ± 0.9 mm; FIBTEM A10 of 14 ± 0.8 and 14 ± 0.9 mm; while FIBTEM LI30-60 were 100% in both groups (Table 7.3). These parameters were not significantly different from baseline values.

***Non-survivors in EPI group*:** After asphyxial-induced cardiac arrest, the non-survivors in EPI group developed relatively similar FIBTEM clotting times (CT, CFT), clot firmness (α, MCF), and clot lysis parameters (LI30-60). Nevertheless, the FIBTEM MCF of normal rats in this study ranged from 12-18 mm, while two out of three rats without ROSC in EPI group had MCF ≥20 mm (data not shown). This led to a mean MCF of 18 ± 2.4 mm, which is somewhat higher than baseline value of 15 ± 0.7 mm. However, the small sample size in the non-survivor group (n=3) was insufficient to reach significance in this study.

7.5 Discussion

Despite ongoing controversy, epinephrine remains the gold standard of resuscitative drugs in cardiac arrest management (Attaran, 2010; Brown*, et al.*, 1996; Gueugniaud*, et al.*, 2000). The present study compared the effects of ALM treatment with epinephrine following 8 min intra-arrest hypothermia (~33^{o}C) and 120 min active rewarming (33-37°C) in the rat model of asphyxial hypoxia.

Epinephrine produced significantly higher arterial pressures during chest compressions than ALM-treated rats. However, three EPI rats failed to attain ROSC compared to 100% attainment in ALM animals. After chest compressions, EPI ROSC achievers generated significantly higher pressures (SAP, DAP, MAP) in the first 5 min compared to ALM treatment. However, form 90 to 120 min the ALM group had developed significantly higher SAP, DAP, MAP than EPI-treated rats. A surprising result was epinephrine-treated rats also exhibited reduced coagulopathy, similar to ALM rats. These results will now be discussed.

7.5.1 Arterial pressures during compressions and ROSC achievement in EPI and ALM-treated rats

In direct contrast to 0.5 ml bolus ALM-treated rats, a 0.5 ml bolus of 0.01 mg/kg epinephrine dramatically increased arterial pressures during chest compressions after asphyxial hypoxia. To put these differences into physiological perspective, the arterial pressures in the EPI group went from near zero during arrest to a systolic/diastolic pressure ratio of 159/114 mmHg for a MAP of 129 mmHg, whereas the ALM treatment went from near zero to 59/29 mmHg for a MAP of 39 mmHg immediately following chest compressions (t = 0, Fig 7.3) for 100% achievement of ROSC. Neumar and colleagues also showed high developed pressures with epinephrine following asphyxial hypoxia (Neumar*, et al.*, 1995). They showed a dose-dependent increase in arterial pressures when epinephrine (0.1 and 1.0 mg/kg) was administered at the onset of CPR, and rats exhibited prolonged post-ROSC hypertension and metabolic acidaemia, increased A-V O_2 gradient, and increased incidence of post-ROSC ventricular tachycardia or fibrillation (Neumar*, et al.*, 1995).

In the present study, as in Chapter 6, no further compressions were administered after the first set and ROSC was achieved. Despite the vasopressor power of epinephrine, three of 12 (75%) EPI animals failed to achieve ROSC due to protracted VF whereas

100% ALM treated animals achieved ROSC. In 2006, McCaul and colleagues also reported that 0.01 mg/kg epinephrine injected into rats following brief asphyxial cardiac arrest was associated with increased mortality with 4 out of 12 failing to achieve ROSC (~33%) (McCaul*, et al.*, 2006b). In addition to asphyxial cardiac arrest, Tang et al. (1995) further showed that in the rat VF model, epinephrine decreased duration of survival and significantly increased the severity of post-resuscitation myocardial dysfunction.

7.5.2 Early post-ROSC haemodynamics: Possible advantages of low, ROSC-generating pressures in the ALM group

Remarkably increased arterial pressures after epinephrine treatment did not guarantee ROSC achievement in this study, as 3 out of 12 epinephrine-treated rats failed to achieve ROSC (Table 7.2). In contrast, although ALM-treated rats had significantly lower arterial pressures during CPR, all of them achieved ROSC.

The advantage of an ALM-induced low-pressure escalation during CPR along with ROSC attainment may be cardioprotective and reduce reperfusion injury. Rea et al (2008) proposed that low-flow from CPR may limit reperfusion injury from post-ischaemic conditioning by attenuating peak levels of oxidative substrate and activating pathways that protect against oxidative stress. Rea and colleagues further proposed that CPR may be considered a dose-sensitive therapy whereby certain physiologic states such as asphyxial hypoxia may benefit from different levels of circulation, and hence, distinct grades of CPR (Rea*, et al.*, 2008). The present study using ALM versus epinephrine would support this proposal. The clinical significance lies in understanding the balance between the low pressures and matching tissue oxygen delivery with the demand among the vital organs during resuscitation. ALM has been shown to resuscitate the MAP into the hypotensive range following severe haemorrhagic shock (Letson & Dobson, 2011a) and associated with a decrease in whole body oxygen consumption, which may ensure the balance of oxygen supply/demand (Granfeldt*, et al.*, 2014; Granfeldt*, et al.*, 2012). Further experiments are required to determine the mechanisms involved and the role of the heart and arterial coupling in providing this protection.

7.5.3 Post-resuscitation haemodynamics in EPI compared to ALM-treated rats

The main problem with EPI is that it is a double-edged sword (Arntz & Breckwoldt, 2012). On one hand, epinephrine increases blood flow to the myocardium (Paradis*, et al.*, 2002) and vital organs (Paradis*, et al.*, 1990; Reynolds*, et al.*, 2010), but on the other hand, it increases myocardial oxygen consumption (Ditchey & Lindenfeld, 1988; Lindner*, et al.*, 1990), increases post-resuscitation myocardial dysfunction (Angelos*, et al.*, 2008; Berg*, et al.*, 1994; Lindner*, et al.*, 1991; Tang*, et al.*, 1995) and is proarrhythmic by shortening action potential refractoriness (Tovar & Jones, 1997). In addition, epinephrine reduces cerebral perfusion during CPR (Ristagno*, et al.*, 2007; Ristagno*, et al.*, 2009a) and induces ischaemia in under-perfused organs (e.g., GI tract and bowel) (Fries*, et al.*, 2006; Rivers*, et al.*, 1994). The current clinical value of epinephrine administration for resuscitation appears to depend on the dosage given, the timing of administration, and the initiating factor of the cardiac arrest (Callaway, 2013).

As mentioned, ALM-treated animals were resuscitated and attained ROSC in a profoundly hypotensive state compared to EPI-treated rats (t=0, Fig 7.3). This occurred along with a significantly lower rate-pressure product, which is an index of myocardial oxygen consumption (White, 1999). The higher rate-pressure product generated after resuscitation with epinephrine may indicate increased myocardial oxygen consumption, supporting the studies of Ditchey and Lindenfeld (1988) and Lindner and colleagues (1990). In the ALM group during the first 5 min after ROSC (Fig. 7.4B). However, at 30 min post-resuscitation, the arterial pressures in the ALM treated rats were not significantly different from EPI-treated rats (Fig. 7.3). The improvement in ALM pressures continued, and from 105 min to 120 min, SAP, DAP and MAP were all significantly higher in the ALM compared to the EPI-treated rats. At this point (105-120 min), the rate-pressure product of the ALM group that of epinephrine, mostly due to a significant drop in epinephrine SAP (see Fig 7.4B and 7.3A). Heart rate was not significantly different between the groups.

Despite its initial vasopressor effects, EPI-treated rats lost their ability to maintain arterial pressures during the two hour post-ROSC period. Neumar et al. (1995) also reported reduced post-ROSC haemodynamics with epinephrine, and showed a progressive decline after 30 min of ROSC compared to placebo. Chen*, et al.* (2010) observed the tendency of MAP to decrease within 60 min ROSC following low, mid, and high doses of epinephrine. Interestingly, it is shown that even a low dose of epinephrine (0.01 mg/kg) decreased short term survival compared to placebo (67% vs 100%) after a brief asphyxia (~4 min) (McCaul*, et al.*, 2006b). Future studies are

required to evaluate ALM versus EPI treatments with a full echocardiographic analysis of chamber volumes, cardiac dynamics, peripheral vascular resistance and blood flow analysis to the heart, lung, brain and vital organs.

7.5.4 Coagulation profiles after cardiac arrest with EPI and ALM treatments

7.5.4.1 Non-ROSC EPI-treated rats were hyperfibrinolytic

Non-ROSC rats from EPI group (t< 5 min) experienced prolonged INTEM CT (and aPTT) and hyperfibrinolysis shown by significantly lower EXTEM LI30-60 and INTEM LI30-60 to a lesser extent. These results were similar to non-ROSC saline controls. Hyperfibrinolysis immediately after asphyxial cardiac arrest has not been reported before in the rat model. The present data support the two clinical studies showing hyperfibrinolysis in out-of-hospital cardiac arrest without the presence of massive blood loss (Schöchl*, et al.*, 2013; Viersen*, et al.*, 2012).

The mechanisms for hyperfibrinolysis are not known, however, may be related to no- or low-flow hypoxic state and hypoperfusion-activated endothelium (Yan*, et al.*, 1999), which in turn stimulate protein C activation (Brohi*, et al.*, 2007b). Apparently the activation of protein C is only found at hospital admission (Adrie*, et al.*, 2005), which may help explain why hyperfibrinolysis in the present study only appeared early in non-ROSC rats (t<5 min from the onset of resuscitation) and was not present after 2 hour of ROSC (Table 7.3). Further work is required to distinguish changes in coagulopathy between non-ROSC and ROSC animals and their possible mechanisms.

Unlike non-ROSC saline-treated rats, two out of three rats in non-ROSC epinephrine group had MCF ≥20 mm, which was above the baseline range (12-18 mm). This may indicate increased fibrinogen level (Schöchl*, et al.*, 2010) early after epinephrine injection, at least in the non-ROSC rats. Elevated fibrinogen level by epinephrine has been demonstrated in rats subjected to tissue injury (Palma*, et al.*, 1981) or during *in vitro* biosynthesis (Roy*, et al.*, 1989). Increased fibrinogen level may shorten clotting times and increase clot firmness, and thus, may prevent hypocoagulopathy (Schochl*, et al.*, 2010). However, precaution is warranted as high level of plasma fibrinogen appears to be an independent predictor of cardiac death in MI patients (Retterstol*, et al.*, 2001). Further study is required to elucidate fibrinogen levels after resuscitation with epinephrine and its possible role in coagulation status following cardiac arrest.

7.5.4.2 Small-volume ALM and EPI bolus treatments show prevention of hypocoagulation compared with saline controls at 120 min

Data from Chapter 6 showed the presence of a hypocoagulopathy in saline controls at 120 min in both plasmatic PT and aPTT values, and in whole blood ROTEM analysis. It was further shown that small-volume ALM prevented PT and EXTEM abnormalities and partially blocked aPTT and INTEM hypocoagulopathy. The present chapter compared the ALM data to epinephrine treatment.

A most interesting result in the present chapter was that EPI-treated animals had the same effect as ALM to prevent extrinsic pathway coagulopathy after 120 min resuscitation (Table 7.3). Under identical experimental conditions, epinephrine treatment led to similar PT (Fig. 7.5) and EXTEM clotting times (CT, CFT), clot firmness (MCF, A5-30), and clot elasticity (MCE) to the baseline values after 120 min of ROSC (Table 7.3). In addition, comparable to ALM, the EPI group did not entirely prevent intrinsic pathway coagulopathy, i.e. the INTEM CTs were 75% and 50% prolonged respectively (Table 7.3). Although due to high standard error means, these prolonged clotting times were not significantly different from baseline (Table 7.3). It would be interesting to compare these indices at 5 min, 30 min, 60 min and over longer periods of resuscitation (6 hours, 24 hours and 36 hours), to see whether intrinsic pathway coagulation parameters return to baseline and, if so, how soon this occurs after resuscitation.

7.5.4.3 Possible mechanisms for prevention of prolonged extrinsic pathway clotting times in EPI-treated animals compared to saline controls

It appears that an epinephrine prevention of hypocoagulopathy two hours post-ROSC compared to saline controls in the present study is a new finding. The possible mechanisms by which epinephrine may decrease clotting times via the extrinsic pathway are not known. Before discussing possible mechanisms, a brief summary of the role of the extrinsic pathway in the clotting cascade is relevant.

Extrinsic and intrinsic pathways of whole blood clotting:

The **extrinsic pathway** is the main pathway for **initiation** of coagulation via Tissue Factor (TF) and Factor VII, whereas the **intrinsic pathway** is more involved during **amplification and propagation** (but not during initiation) of the coagulation process (see Fig 7.6) (Mackman*, et al.*, 2007). The extrinsic pathway is a very rapid “initiator” of

the clotting process that occurs in 10 sec to 4 min, and results in small amounts of thrombin (in nanomolar concentrations) (Brummel*, et al.*, 2002; Monroe & Hoffman, 2006). In contrast, the spatially separate intrinsic pathway takes 5-16 min to generate larger amounts of thrombin (>96%) for a stronger, longer and larger clot during the propagation phase of coagulation (Brummel*, et al.*, 2002; Monroe & Hoffman, 2006). Both pathways are involved in clot formation since TF-VIIa complex from the extrinsic pathway and IXa-VIIIa complex from the intrinsic pathway convert Factor X to Xa in a single **common pathway**, which in turn forms Factor Xa-Va complex and activates thrombin (see Fig. 7.6). Thrombin cleaves fibrinogen into fibrin, which is cross-linked by XIIIa/Transglutaminase to form the fibrin clot.

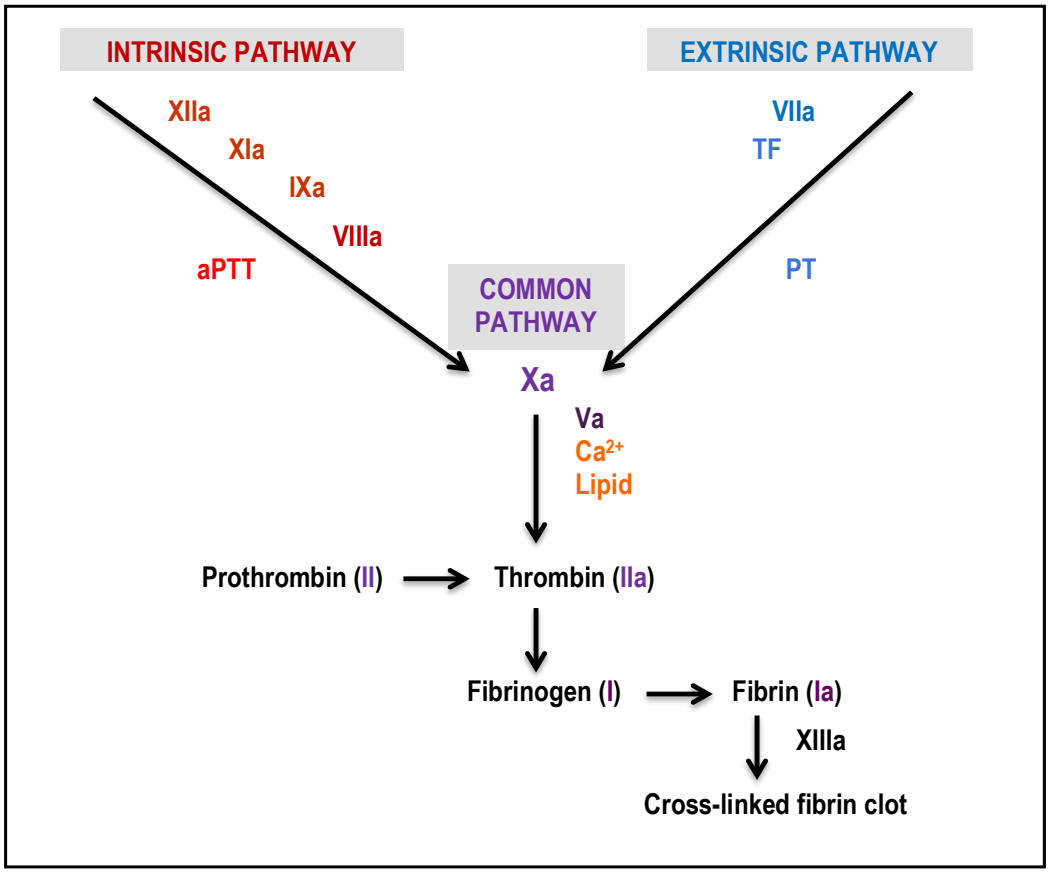


Figure 7.6 Simplified coagulation cascades illustrating the role of extrinsic and intrinsic pathways in clot formation
The extrinsic pathway involves Tissue Factor (TF) and Factor VIIa complex activation. The intrinsic pathway involves activation of Factor XIIa, XIa, IXa and VIIIa. Both extrinsic and intrinsic pathways serve to activate common pathway factor Xa, which in turn converts prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin and activates factor XIIIa to stabilise fibrin clot. Prothrombin time (PT) assesses coagulation factors in **blue**, activated partial thromboplastin time (aPTT) assesses factors in **red**, while both measure factors in **purple** (common pathway).

Prolongation of PT and aPTT and EXTEM and INTEM CT in saline controls

Since the **extrinsic pathway** involves Tissue Factor and Factor VII complex activation of Factor X before entering the common pathway of clot formation, a prolongation of PT in saline controls implies a deficiency or inhibition of Factor VII, X, V, II and/or fibrinogen. Similarly, a prolonged aPTT indicates abnormalities of all the clotting factors of the intrinsic and common pathway with the exception of Factor VII. Prolongation of both PT and aPTT could involve any or all of the above factors of the extrinsic, intrinsic, and common pathways, including reduced fibrinogen availability or inhibition, which would effectively increase bleeding.

Prevention of prolonged extrinsic pathway clotting times by epinephrine

Since epinephrine significantly reduced PT and EXTEM clotting times at two hours post-ROSC compared to saline controls, it is concluded that epinephrine must exert its effect at one or more reactions involving increasing the availability of Tissue Factor, Factor VII or reactions of the common pathway Factor X, Factor V and/or activation of fibrinogen.

Epinephrine has been known to influence coagulopathy for over a century. In 1903, Vosburgh & Richards showed that adrenalin (epinephrine) shortened whole blood-clotting times when administered intra-peritoneally in dogs (Vosburgh & Richards, 1903). Cannon and Gray (1914) also found a similar shortening effect of 30 to 50% of control values in cats from a 1.0 µg/kg intravenous epinephrine injection. The effects of epinephrine appear to be dose dependent as higher doses (up to 80 µg/kg) caused prolongation of clotting, not shortening (Forwell & Ingram, 1957; Wenzel & Singh, 1962). It is curious, however, in this present study, how a small-volume epinephrine bolus with a half-life of around a few minutes in blood had such a profound effect on shortening PT and EXTEM times at 2 hours compared to saline controls.

While the mechanisms of epinephrine's effect to shorten clotting times are not fully understood, some studies indicated that epinephrine stimulate increased platelet aggregation in humans and rabbits (Mills & Roberts, 1967; Wenzel & Singh, 1962), increased fibrinogen biosynthesis and plasma level in injured rats (Palma*, et al.*, 1981; Roy*, et al.*, 1989), and increased platelet-fibrinogen binding in humans (Hjemdahl*, et al.*, 1994) and fibrinolytic (Chandler*, et al.*, 1992). In addition, intravenous infusion of epinephrine caused a significant rise in Factor VIII (Ingram & Jones, 1966; Prentice*, et al.*, 1972), and Factor V activity in resting humans (Forwell & Ingram, 1957). Factor

VIII, however, is part of the intrinsic pathway and unlikely to be involved in shortening the PT or EXTEM clotting times. Activating Factor V is a potential site in the common pathway that is a possibility for epinephrine's procoagulant properties, but Forwell and Ingram make no further mention of this effect in any of their subsequent publications after it was first published in 1957 (Forwell & Ingram, 1957).

In summary, the present study was among the first to show a prevention of acute hypocoagulopathy in EPI-treated rats involving the extrinsic pathway. Unfortunately, despite epinephrine effects on blood coagulation factors being studied since 1903 (Vosburgh & Richards, 1903), there is inadequate literature on epinephrine effects on coagulation following experimental or clinical cardiac arrest. Further studies are required to elucidate the mechanisms of acute coagulopathy post-arrest and resuscitation and those protective mechanisms of epinephrine compared to saline controls. The timing of coagulation changes immediately after ROSC, as well as for hours afterwards, warrant further investigation, and a complete profile of the clotting factors, platelet analysis, thrombin levels, fibrinogen and D-dimers following asphyxia-induced cardiac arrest is required.

7.5.4.4 ALM prevention of acute hypcoagulopathy

Similar to EPI, ALM treatment resulted in approximately normal values for PT, all EXTEM and most INTEM clotting parameters after 120 min post ROSC. The possible mechanisms for ALM protection against coagulopathy are not known and were discussed in Chapter 6. There may be some similarities to the effects of epinephrine, but the mechanism involved in the coagulation restoration by ALM may also related to its anti-inflammatory effects. Previously, the AL combination has been shown to possess superior anti-inflammation compared to adenosine or lidocaine alone, including *in vitro* inhibition of platelet activating factor–induced CD11, CD18 expression, and PMN adhesion (Shi*, et al.*, 2012). The anti-inflammatory effects of ALM or AL may prevent acute coagulopathy by: 1) concurrently improving altered coagulation, since inflammatory mediators can over-activate coagulant proteins, inhibit endogenous anticoagulant proteins and suppress fibrinolytic factors (Esmon, 2005), and/or 2) attenuating endothelial injury, and hence, possibly reducing consequent coagulopathy-induced by endothelial activation following cardiac arrest (Adrie*, et al.*, 2005; Böttiger*, et al.*, 2002).

7.6 Limitation of the study

The present study compared ALM and EPI effects on haemodynamics and coagulopathy post cardiac arrest. However, intra-arrest hypothermia and active rewarming applied in this study may confound the interpretation of ALM or EPI post-resuscitation haemodynamics and coagulation functions. Again, two-hour observation period may also limit the ability to examine EPI or ALM's effects on post-ROSC haemodynamic instability or death.

7.7 Conclusion

A 0.5 ml bolus of 0.01 mg/kg epinephrine dramatically increased systolic/diastolic pressure to 159/114 for a MAP of 129 mmHg for 75% ROSC attainment. Death (n=3) was from VF. In contrast, ALM treatment led to pressures of 59/29 for a MAP of 39 mmHg for 100% achievement of ROSC. After 15 min ALM-treated animals changed from a hypotensive state with continual increases in pressures, and after 90 min, the SAP, DAP and MAP were all significantly higher in the ALM- than the epinephrine-treated rats. Epinephrine and ALM treatments prevented acute coagulopathy at 120 min in both extrinsic PT and EXTEM clotting times compared to saline controls.

CHAPTER 8. GENERAL DISCUSSION

8.1 Restatement of the problem and general aim

More than a million cardiac arrest incidences are documented in developed countries every year, primarily occurring in out-of-hospital settings (Ramaraj & Ewy, 2009). In the United States alone, cardiac arrest claims over 400,000 adult lives and around 16,000 children each year (Roger*, et al.*, 2012; Tress*, et al.*, 2010). The two most prevalent causes of sudden death are of cardiac and respiratory origins (asphyxia) (Moriwaki*, et al.*, 2013; Tress*, et al.*, 2010). Although myocardial dysfunction after cardiac arrest from VF has been extensively studied, less is known about heart function and haemodynamics following asphyxial cardiac arrest (George, 2013; McCaul*, et al.*, 2006b). Failure to adequately rescue the heart, brain and vascular system leads to post-resuscitation syndrome, which comprises inflammatory and coagulation imbalances, organ failure and death (Adrie*, et al.*, 2005; El-Menyar, 2005; Nolan*, et al.*, 2008). The biggest killer is time as the chance of survival in adults declines by up to 5.2% for every minute of no-flow status (Gold*, et al.*, 2010). Currently, there is no rescue drug therapy that improves cardiac arrest survival in the adult or paediatric population (Ewy & Sanders, 2013; Nolan*, et al.*, 2012; Olasveengen*, et al.*, 2009b; Tress*, et al.*, 2010).

In an effort to translate the cardioprotective effects of adenosine and lidocaine (AL) from cardiac surgery (Dobson*, et al.*, 2013) and trauma (Granfeldt*, et al.*, 2014; Letson & Dobson, 2011a) to cardiopulmonary resuscitation, the main aim of this thesis was to investigate the effect of adenosine (A), lidocaine (L), AL and AL with $MgSO_4$ (ALM) on cardiovascular rescue dynamics, arrhythmias and coagulopathy following eight minutes of asphyxial hypoxia in the rat model during normothermia and mild-to-moderate hypothermia. A small-volume bolus of ALM was then compared with the current standard-of-care, epinephrine. The main results of this thesis will now be discussed.

8.2 Major findings

The main findings of the thesis were:

1) In Chapter 3, the optimal bolus dose of AL for ROSC attainment and haemodynamic function during chest compressions was found to be 0.48mg/1.0 mg adenosine/lidocaine in a total volume of 0.5 ml. With body temperature drifted to 34-35°C (accidental hypothermia), a single bolus of AL immediately prior to chest compressions was shown to improve developed haemodynamics (SAP, MAP, DAP ($p<0.05$)) during compressions compared to adenosine, lidocaine and saline, with lower heart rates than saline controls. AL treatment also resulted in a more relatively stable ECG rhythm during compressions and hands-off periods. However, no group could sustain ROSC after 5 to 10 min resuscitation at body temperatures ranging from 34 to 35°C.

2) In Chapter 4, actively warming to maintain normothermia confirmed the finding in Chapter 3 (accidental hypothermia) that no group could maintain ROSC after 5 to 10 min regardless treatment given. In contrast, cooling the animal during cardiac arrest and for 60 min post-resuscitation (28-33°C) significantly improved ROSC attainment and haemodynamic function during intermittent chest compressions in all treatment groups. During hypothermia, after 30 min of intermittent chest compressions, no saline controls compared to two of eight (25%) lidocaine-treated rats and six of eight (75%) AL-treated rats achieved ROSC. It was concluded from *Kaplan-Meier* analysis that during mild-to-moderate hypothermia, AL was significantly superior in attaining ROSC following intermittent chest compressions over 60 min.

In the second part of Chapter 4, it was shown that after one 60 sec set of chest compressions, 100% of AL-treated cold animals could generate ROSC and sustain it for 60 min compared to seven out of eight (87.5%) lidocaine-treated rats and six out of eight (75%) saline controls. The AL-treated rats, but not lidocaine-treated rats, had significantly lower HR than saline controls immediately at ROSC ($t=0$) and over 60 min post resuscitation ($p<0.05$ at 45 and 55 min). It was concluded that mild-to-moderate hypothermia was a powerful strategy to improve ROSC sustainability in the rat model of 8 min of asphyxial hypoxia.

3) In Chapter 5, the effect of adding magnesium sulphate ($MgSO_4$) to adenosine (AM), lidocaine (LM) and AL (ALM) on ROSC, haemodynamics and coagulopathy was examined during two hour induced moderate hypothermia (28-32^{o}C). It was shown that resuscitation with AL and ALM injection led to 100% ROSC compared to 87.5% for LM, 50% for AM, 62.5% for Mg alone, and 75% for saline controls. ALM treatment with moderate hypothermia also significantly improved haemodynamics from 30-120 min post-ROSC compared to AL, AM, LM and Mg and saline groups. AL and ALM had no VF, and AL had the lowest incidence of VT compared to any other group. In addition, ALM, but not AL, protected against coagulopathy (prolonged PT and aPTT) after two hours of ROSC.

4) Chapter 6 showed that after intra-arrest hypothermia (~33^{o}C) followed by immediate active rewarming (33-37°C): 1) ALM resuscitation significantly improved ROSC attainment with significantly higher MAPs from 75-120 min compared to saline controls; 2) In contrast to saline controls, ALM prevented a number of coagulation abnormalities associated with cardiac arrest, except for prolongation of INTEM CT; and 3) ALM did not significantly reduce the early neurological changes in neocortex and hippocampus compared to saline controls as assessed by routine histological methods. It was concluded that there appeared to be no deleterious effect of actively rewarming the animal after hypothermia on ALM's ability to resuscitate MAP or its effect to protect against coagulopathy.

5) In Chapter 7, a single bolus of ALM was compared to a single bolus of standard-of-care epinephrine (0.01 mg/kg) using the same temperature protocol described in Chapter 6. It was found that immediately upon resuscitation, epinephrine dramatically increased systolic/diastolic pressure to 159/114 for a MAP of 129 mmHg for 75% ROSC attainment. In contrast, all ALM-treated animals achieved ROSC at significantly lower pressures of 59/29 for a MAP of 39 mmHg. From 90 min to 120 min, the ALM group developed significantly higher systolic and diastolic pressures, significantly higher MAP and significantly higher rate-pressure, an index of myocardial oxygen consumption product, than EPI-treated rats. Another interesting result was that, like ALM rats, epinephrine-treated rats also prevented hypocoagulopathy at 120 min compared to saline controls.

8.2.1 The effects of adenosine, lidocaine, and AL on haemodynamics and ECG rhythm during intermittent chest compressions

Following cardiac arrest, high quality chest compressions during cardiopulmonary resuscitation (CPR) are essential to re-establish coronary and cerebral blood flow; however, chest compressions at best can only provide 10 to 20% of normal coronary perfusion and 20 to 40% of normal cerebral blood flow, with almost no forward flow to the periphery (Gazmuri & Becker, 1997; Kern, 2000; Rubertsson & Karlsten, 2005; Yannopoulos*, et al.*, 2005). Since cardiac (and cerebral) failure or dysfunction are largely responsible for the high mortality (>90%) and morbidity following cardiac arrest (El-Menyar, 2005; Lemiale*, et al.*, 2013; Stub*, et al.*, 2011), any new drug therapy that could improve developed pressures and haemodynamic stability *during chest compressions* may improve outcomes.

The administration of adenosine and lidocaine (AL) during resuscitation attempts led to higher arterial pressures during chest compressions with 5 min intervals up to 60 min compared to saline or each drug alone (see Chapter 3). After 30 min of the intermittent chest compressions, only AL treatment could produce DAPs of 12-15 mmHg, while control and adenosine DAPs were <5 mmHg (see Fig.3.4 B). It is suggested that higher developed pressures in AL-treated animals may occur from a more compliant or distensible myocardium permitting more blood to enter its chambers during each decompression phase, and therefore, facilitating haemodynamically efficient chest compressions (Gazmuri & Radhakrishnan, 2012). The higher DAPs during CPR with AL bolus could be essential for two reasons: 1) it may increase coronary blood flow to the globally ischaemic heart, since aortic diastolic pressure is a major determinant of coronary perfusion at this stage (Frenneaux & Steen, 2007), and 2) it may reflect a higher peripheral vascular tone with improved forward flow to vital organs (Chamberlain*, et al.*, 2008; Kern, 2000).

Another key finding from the study presented in Chapter 3 was that AL treatment also led to more stable ECG rhythms during chest compressions. This is shown by incredibly small standard error means (SEM) of the R-R interval of the ECG recording during chest compressions compared to the other treatment groups (Fig. 3.6B), and no fatal arrhythmias (VF) experienced in AL-treated rats (Table 3.7). This could be critical because chest compression itself can stimulate ventricular electrophysiological instability (Osorio*, et al.*, 2008), which can lead to recurrent VF as shown in ~70% OHCA patients during compressions (Berdowski*, et al.*, 2010). A more stable ECG

(rhythm and rate) with AL treatment was also identified in a rat model of myocardial infarction, with 90% reduction in ventricular arrhythmias compared with adenosine or lidocaine alone (Canyon & Dobson, 2004). In addition, 7.5% NaCl AL with Mg^{2+} treatment also prevented ventricular arrhythmias in a near-lethal rat model of 60% blood loss and 60 minute shock, compared with 7.5% NaCl controls (Letson & Dobson, 2011b).

8.2.1.1 Accidental hypothermia versus normothermia: The heart generates higher pressures during chest compressions when the animal's body temperature is allowed to drift over one hour

Chapter 3 showed that during accidental hypothermia no treatment group (adenosine, lidocaine or AL) or saline controls could sustain or generate ROSC after 5 min (Table 3.5). This result was also observed during active warming normothermia (Chapter 4, Fig. 4.2). The first question that was addressed in this thesis was how does adenosine, lidocaine and AL combination compare in rescuing the haemodynamics *during chest compressions* without ROSC being achieved. Over a 60 minute period twelve sets of 30 sec manual compressions were performed at 300 beats per min (confirmed by MacLab) with 5 min hands-off periods. While all current guidelines emphasize minimal interruptions during chest compressions (Deakin*, et al.*, 2010; Ewy & Sanders, 2013; Neumar*, et al.*, 2010; Tibballs*, et al.*, 2012), the hands-off periods in this thesis allowed the assessment of different drugs on haemodynamics and ECG rhythms during each compression phase over a one-hour resuscitation period.

During accidental hypothermia, it was shown that AL led to higher SAP, DAP ($P<0.05$) and MAP compared to any other group during chest compressions (Fig 3.4). Again, it is possible that AL's improved diastolic function indicates improved left ventricular (LV) myocardial distensibility or improved LV wall thickening and slowing haemodynamic decay during prolonged, intermittent chest compressions (Ayoub*, et al.*, 2003; Gazmuri*, et al.*, 2008; Klouche*, et al.*, 2002). In contrast, during normothermia, these group treatment differences disappeared, although AL and lidocaine haemodynamic measurements remained at least 1.7 times higher than saline controls in the first 15 min (Fig 4.3). An adenosine alone group was not included in the normothermia experiments because of the high mortality (50%) and high incidence of severe arrhythmias observed during accidental hypothermia (Chapter 3, Table 3.5).

It is interesting that accidental hypothermia and normothermia led to different developed haemodynamics in treatment groups. During normothermia, the heart's ability to develop arterial pressures during chest compressions after 15 min post-drug injection was significantly less compared to if the animal's body temperature was allowed to spontaneously drift to ~34°C (Fig. 8.1). For example, for AL-treated animals at t=15 the SAP/DAP was 26/19 mmHg for a MAP of 21 mmHg during accidental hypothermia, compared to 14/13 mmHg for a MAP of 13 mmHg during normothermia. Similarly at 30 min of resuscitation, the developed pressures were 19/15 mmHg for a MAP of 16 mmHg during accidental hypothermia, and 6/5 mmHg for a MAP of 6 mmHg during normothermia. As resuscitation time increased, the differences became smaller between groups, however, pressures in the normothermic groups were still lower than accidental hypothermic groups at any time point, particularly those treated with AL or lidocaine (Fig. 8.1). Mild heat loss in the accidental hypothermic rats appears to be well tolerated with positive cardiovascular outcomes following cardiac arrest. It was concluded that no active warming and allowing the body temperature to drift during arrest and resuscitation had a significant positive effect on the ability of the heart to generate pressures *during chest compressions*, with no associated improvement in the incidence of ROSC. Within homeostatic limits, accidental or spontaneous hypothermia may be a pro-survival strategy, and the animal's way of defending itself against the initial trauma of cardiac arrest. Further studies are required to explore this difference between accidental hypothermia and normothermia on cardiac and brain functions in the rat model of asphyxial hypoxia.

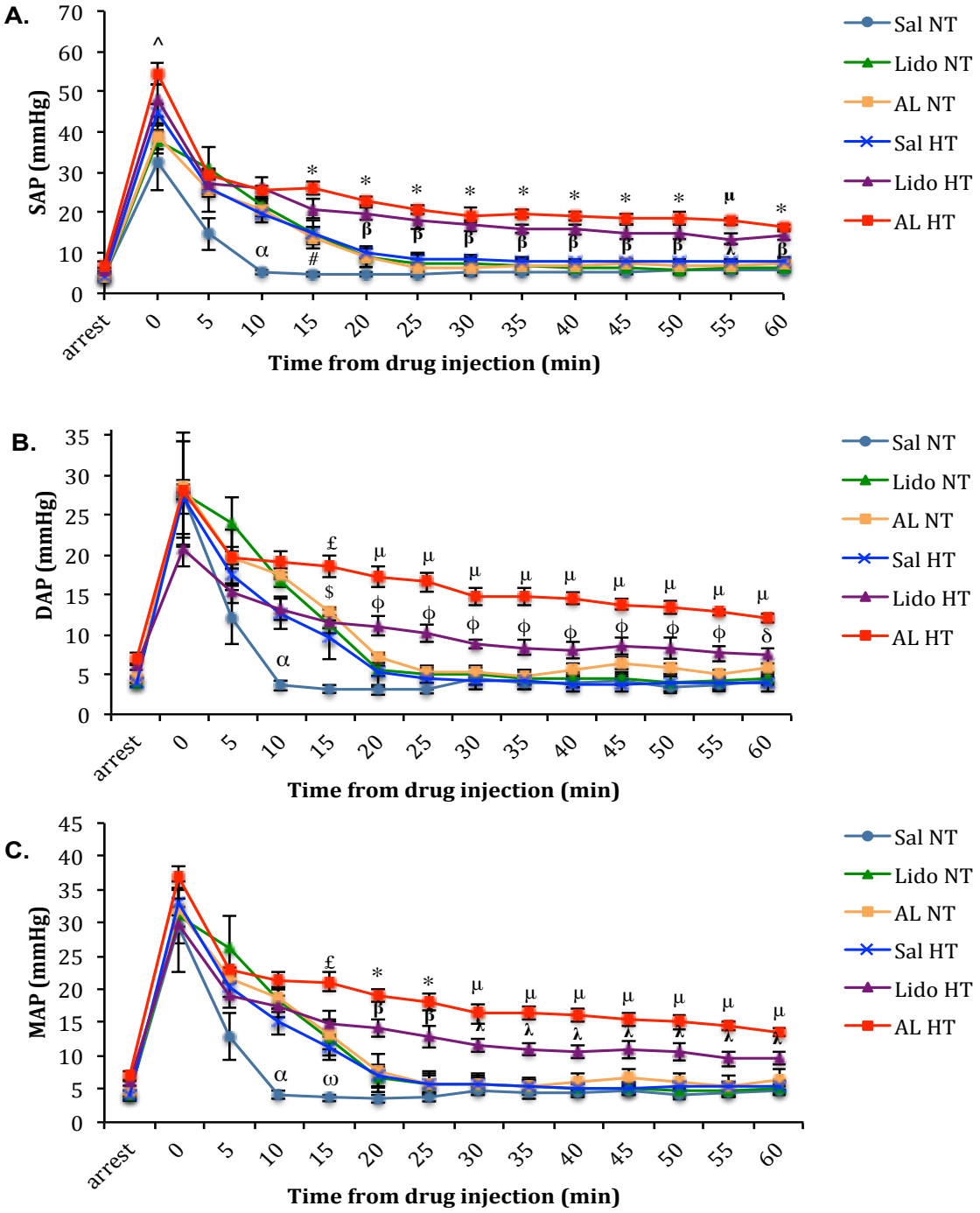


Figure 8.1 A. Systolic arterial pressures (SAP), B. Diastolic arterial pressures (DAP), and C. Mean arterial pressures (MAP) during 30 sec of compressions during normothermia (NT) and accidental hypothermia (HT) between treatment groups

Values represent mean ± SEM. ^p<0.05 for Sal NT compared to AL HT. αp<0.05 for Sal NT compared to all other groups. #p<0.05 for Sal NT compared to all other groups except AL NT. *p<0.05 for AL HT compared to all other groups except Lido HT. βp<0.05 for Lido HT compared to all other groups except AL HT. £p<0.05 for AL HT compared to Sal NT and Sal HT. $p<0.05 for AL NT compared to Sal NT. µp<0.05 for AL HT compared to all other groups. λp<0.05 for Lido HT compared to all other groups. Φp<0.05 for Lido HT compared to all other groups except AL NT. δp<0.05 for Lido HT compared to all other groups except AL NT. ωp<0.05 for Sal NT compared to all other groups except Sal HT

8.2.1.2 Effect of mild to moderate hypothermia with AL and lidocaine treatment on ROSC achievement and ECG stabilisation

While therapeutic hypothermia is recommended by international guidelines, the timing, extent and duration remain controversial (Lindner*, et al.*, 2013; Sunde & Søreide, 2011). There is a growing clinical consensus that therapeutic hypothermia during cardiac arrest and newborn asphyxia has positive neurological outcomes (Holzer, 2013; Jacobs*, et al.*, 2011b) as well as survival to hospital discharge (Hinchey*, et al.*, 2010; Reinikainen*, et al.*, 2012). However, it must be kept in mind that the survival rate for all forms of out-of-hospital cardiac arrest still remains <10% (McNally*, et al.*, 2011; Sasson*, et al.*, 2010). Thus, hypothermia, although promising, does not appear to be the major solution to improve survival and it will likely involve innovations involving hypothermia, improved pharmacotherapies and new modalities of pre-hospital and post-operative care.

Chapter 4 showed that inducing mild-to-moderate hypothermia over the entire experimental period (asphyxial arrest and 60 min resuscitation) was very effective and significantly improved the incidence of ROSC and haemodynamics during intermittent chest compressions in all treatment groups and saline controls. Furthermore, during hypothermia after one 60 sec set of compressions, 100% of AL-treated compared to 87.5% Lido-treated rats and 75% saline controls could generate ROSC and sustain it for 60 min (see Fig. 4.5), while none of the normothermic groups could sustain ROSC for more than 5-10 min. Early application of hypothermia in this study may provide greater cardio- and neuro-protection through two different windows of opportunity. First, during arrest or no-flow, hypothermia down-regulates cellular metabolism (Yenari*, et al.*, 2008), preserves cellular function (Carrier*, et al.*, 1994; Ning*, et al.*, 1998), reduces toxic metabolites and reactive oxygen species generation, and inhibits intracellular Ca^{2+} overload (Chin*, et al.*, 2007; Gundersen*, et al.*, 2001; Sakoh & Gjedde, 2003). Second, during reperfusion, hypothermia modulates necrotic and apoptotic pathways, attenuating inflammatory reactions and preventing cellular death (Holzer*, et al.*, 2005).

Interestingly, only AL treatment with induced hypothermia could increase the chance of ROSC compared to lidocaine alone or saline in rats that received intermittent chest compressions. Although both AL and lidocaine treatment in cold increased the chance of ROSC, the number of rats that achieved ROSC in the AL group was three times higher than the lidocaine group after 30-40 min of prolonged intermittent chest

compressions (Fig. 4.2A). Intermittent chest compression in this study may resemble poor quality CPR with prolonged interruptions, which is associated with worse resuscitation outcomes in clinical practice (Kern, 2003; Valenzuela*, et al.*, 2005).

Another recurring theme was that AL led to no VF compared to all other groups, regardless of the temperature (Table 4.2). Although lidocaine treatment relatively reduced the incidence of VF compared to saline controls, two and one animals during warm and cold lidocaine resuscitation, respectively, did present with VF (Table 4.2). Lidocaine alone was a powerful anti-arrhythmic nonetheless. Despite it being the drug of choice in the 1980s for cardiac arrest victims on location, the lack of clinical support relegated it to a second-line drug after amiodarone (Neumar*, et al.*, 2010). However, two large multicentre trials recently have shown lidocaine to be superior to amiodarone in adults (Pollak*, et al.*, 2006; Rea*, et al.*, 2006), and in another study in younger patients (<18 years old) (Valdes*, et al.*, 2014), and the field is beginning to rethink its clinical use in-hospital. AL may show promise in this area because it did not only reduce arrhythmias during accidental hypothermia and normothermia, but also during mild-to-moderate hypothermia. It is noteworthy that overcooling (<32^{o}C) often occurs in cardiac arrest patients (Gillies*, et al.*, 2010; Merchant*, et al.*, 2006), which is associated with increased risk of ventricular arrhythmias (Polderman & Herold, 2009).

8.2.2 The effects of intra-arrest and post-resuscitation hypothermia on adenosine, lidocaine, and AL with or without Mg^{2+} on haemodynamics, ECG rhythm, and coagulopathy following ROSC

Chapter 5 showed that the addition of magnesium sulphate to AL solution (ALM) significantly improved post-ROSC haemodynamics from 90-120 min compared to AL, AM, LM, $MgSO_4$ alone, and saline groups during induced moderate hypothermia (28-32^{o}C) (Fig. 5.2). This could be clinically important as extremely low body temperature can depress cardiac function leading to systemic hypotension (Moore*, et al.*, 2011). While the mechanism for ALM haemodynamic improvement is still uncertain, Mg^{2+} administration may positively affect myocardial contractility (Nair & Nair, 2000; Rasmussen*, et al.*, 1988) through its influence on intracellular Ca^{2+} level (Agus & Agus, 2001; Reinhart, 1991) and ATP generation (Saris*, et al.*, 2000). However, $MgSO_4$ treatment alone produced the lowest SAP, DAP and MAP after 30 min of ROSC (Fig. 5.2). Thus, the mechanism of adding Mg to AL to improve post-ROSC haemodynamics requires further investigation.

It was also found that ALM and AL anti-arrhythmic properties are versatile even at very low temperature (down to 28^{o}C). Moderate hypothermia (<32^{o}C) could be arrhythmogenic (Ujhelyi*, et al.*, 2001) and was found to increase the risk of arrhythmias in 18% of cardiac arrest patients (Nielsen*, et al.*, 2011). No incidence of VF was experienced in ALM or AL-treated rats compared to four saline controls and one AM-treated rat during two hours of induced moderate hypothermia (Table 5.4). This is crucial for survival since VF could be persistent and resulted in no ROSC in two saline controls (Table. 5.2). However, the endpoints measured and level of analysis in Chapter 5 was not exhaustive enough to conclude that increased risk of arrhythmias was actually derived from moderate hypothermia or as a result of over-excitability of myocytes following cardiac arrest and chest compression.

Another important finding in Chapter 5 was that the addition of Mg to AL solution also prevented the prolonged plasmatic clotting times at 120 min following asphyxial-induced cardiac arrest. Neither Mg^{2+}, AM, LM nor AL treatment was able to prevent the hypocoagulopathy. How ALM can maintain and/or restore coagulation is not fully understood. However, it could be related to ALM-improved post-resuscitation haemodynamics, since hypoperfusion may induce acute coagulopathy as shown in trauma and cardiac arrest patients (Brohi*, et al.*, 2008; Viersen*, et al.*, 2012). Since the coagulation benefit only arose with ALM treatment, the following studies focussed on further elucidating ALM effects on post-ROSC coagulopathy, compared with saline controls (Chapter 6) or the gold standard treatment epinephrine (0.01 mg/kg) (see section 8.2.4).

8.2.3 The effects of intra-arrest hypothermia and post-resuscitation rewarming with ALM and epinephrine on haemodynamics and ECG rhythm following ROSC

During chest compressions, epinephrine dramatically increased systolic/diastolic pressure to 79/64 mmHg but 25% of EPI-treated rats failed to achieve ROSC. In contrast, 100% of ALM-treated rats achieved ROSC at significantly lower developed pressures of 49/39 mmHg (Table 7.2). Immediately upon resuscitation, epinephrine-treated rats had systolic/diastolic pressure of 159/114 mmHg for a MAP of 129 mmHg, whereas ALM rats had 59/29 mmHg of systolic/diastolic pressure for a MAP of 39 mmHg (t=0, Fig 7.3). As shown in previous chapters, ROSC was achieved by ALM rats at significantly lower heart rates (t=0, Fig 7.4A). A lower heart rate for hypotensive ROSC may reflect increased cardiac filling times (Sherwood, 2013), improved stroke volume from increased systolic ejection time (Granfeldt*, et al.*, 2014), and/or reduced

myocardial oxygen consumption (Canyon & Dobson, 2006; Reil & Böhm, 2007). During the last 30 min observation period (30-60 min), ALM-treated rats developed significantly higher arterial pressures and rate-pressure product than EPI-treated rats.

The data showing that ALM was superior to epinephrine may be clinically significant. Epinephrine has been the mainstay for cardiopulmonary arrest since the 1940s (Callaway, 2013). The therapeutic rationale has been to partially restore myocardial and cerebral blood flows by epinephrine's α-adrenergic agonist vasopressor actions on the peripheral vasculatures (Callaway, 2012; Koehler*, et al.*, 1985; Michael*, et al.*, 1984). The increased myocardial perfusion in turn increases coronary perfusion pressures during CPR and the chance of ROSC (Paradis*, et al.*, 1990; Reynolds*, et al.*, 2010). Nevertheless, recent randomised placebo-controlled trials show that although epinephrine administered during CPR may increase ROSC, no differences were found in survival to hospital discharge or neurological outcome (Jacobs*, et al.*, 2011a). Some human registry data suggests that epinephrine may in fact be harmful and decrease survival and worsen neurological outcomes (Hagihara*, et al.*, 2012; Ristagno*, et al.*, 2009a). Hagihara and colleagues (2012) showed that use of prehospital epinephrine increased the chance of ROSC but decreased survival and functional outcomes one month after hospital admission. In a recent review, Callaway argues that prospective clinical trials are required to determine the correct dose, timing and type of patient for continued epinephrine use in cardiac arrest resuscitation (Callaway, 2013).

As mentioned in the introduction and Chapter 7, animal and human studies have shown that epinephrine's benefit appears to arise from its ability to increase aortic pressure and coronary blood flow *during chest compressions,* and therefore improve the likelihood of ROSC (Paradis*, et al.*, 1990; Paradis*, et al.*, 1991; Reynolds*, et al.*, 2010). The negative effects, on the other hand, come from its ability to increase heart rate (chronotropy), ventricular contraction (inotropy) and myocardial oxygen consumption resulting in myocardial supply/demand imbalances and dysfunction post-ROSC (Ditchey & Lindenfeld, 1988; Klouche*, et al.*, 2003). In rats, Tang and colleagues (1995) showed that epinephrine increased the severity of post-resuscitation cardiac outcome. More recently, two separate studies showed that esmolol, a short-acting β1-selective adrenergic blocking agent, given with epinephrine during CPR significantly reduced post-resuscitation myocardial dysfunction in rats (Huang*, et al.*, 2004) and recurrent VF in pigs (Jingjun*, et al.*, 2009). The present thesis would support the negative effects of epinephrine in attaining ROSC and decreasing haemodynamic function after 90 min in the rat model of 8 min asphyxial hypoxia. Further studies are

required to extend these ALM versus EPI comparisons and evaluate *in vivo* cardiac function with echocardiography during early and longer post-resuscitation periods in rat and larger animal models of asphyxial hypoxia.

8.2.4 ALM and epinephrine's possible benefits to correct post-ROSC hypocoagulopathy

Following resuscitation, the immediate post-cardiac arrest period is predominantly characterized by haemodynamic instability (Laurent*, et al.*, 2002), and also inflammatory and coagulation imbalances (Adrie*, et al.*, 2002; Adrie*, et al.*, 2005). Until a few years ago, very little was known about coagulopathy in these low-flow states. In the present study, it was shown that hyperfibrinolysis occurred after 8 min cardiac arrest in the asphyxial hypoxia rat model (Chapter 6). Viscoelastic analysis of whole blood using ROTEM at 120 min post-ROSC, after 0.9% NaCl treatment showed prolonged clotting times, reduced maximum clot firmness and decreased clot amplitudes with no hyperfibrinolysis. It was further shown that ALM prevented these abnormalities with the exception of INTEM CT (intrinsic pathway) at 120 min (Chapter 6). However, the defect in INTEM CT with ALM treatment occurred without measurable consequences on clot amplitude (A5-30) or maximal clot elasticity (MCE). This may confirm the temporal and spatial separation of the extrinsic and intrinsic clotting pathways postulated by Ovanesov and colleagues, with the intrinsic pathway (INTEM) acting to amplify or propagate, but not to initiate, the growth phase of the clot leading to the physical expansion of the fibrin clot (Ovanesov*, et al.*, 2005). Unlike extrinsic factors, the role of intrinsic pathways is not obvious in *in vivo* haemostasis, but its contribution to thrombus formation becomes more important during pathological states (Gailani & Renne, 2007). Abnormality in the intrinsic pathway (INTEM) is associated with reduced thrombus propagation and stability, but less likely to manifest in bleeding disorders (Revenko*, et al.*, 2011).

Similar to ALM, in Chapter 7, it is shown that epinephrine prevented prolongation of PT and EXTEM CT but not aPTT or INTEM CT. Currently the mechanism of coagulation protection is unknown, only that it appears isolated to the extrinsic pathway. However, some literature suggests epinephrine increases coagulation Factor VIII (Ingram & Jones, 1966; Prentice*, et al.*, 1972), which is crucial to the intrinsic pathway, while others suggest epinephrine may increase Factor V (Chesney*, et al.*, 1981; Forwell & Ingram, 1957). The present study did not measure the various individual factors of the intrinsic and extrinsic pathways of coagulation, however, epinephrine's effect on Factor

V is interesting as it is the common pathway and may be responsible for the maintenance of normal PT and EXTEM CT. Again, further investigations are required to test this hypothesis.

Although acute coagulopathy following cardiac arrest has been recognised and independently predicts poor prognosis and death of the victims (Kim*, et al.*, 2013), there are no established strategies to manage post-arrest coagulopathy. In trauma patients, tranexamic acid, fibrinogen concentrate and prothrombin complex concentrate are currently used to manage bleeding and coagulopathy (Napolitano*, et al.*, 2013; Schöchl*, et al.*, 2014). However, the coagulation abnormality in cardiac arrest settings is distinctive. It may involve increased coagulation, reduced anticoagulation and reduced fibrinolysis (Bottiger*, et al.*, 1995) or hyperfibrinolysis (Viersen*, et al.*, 2012), and may vary during arrest, CPR and following reperfusion (White*, et al.*, 2011). Moreover, the derangements could be complicated with cardiac arrest treatment modalities such as therapeutic hypothermia and thrombolytic agents (Weidman*, et al.*, 2014). Finding potential treatment strategies to treat or prevent coagulopathies associated with cardiac arrest, CPR and post-arrest resuscitation may greatly enhance patient outcomes. A small-volume bolus of ALM administered *immediately* prior to chest compressions may help rescue and stabilise the heart of OHCA patients, and combined with ALM protection against coagulopathy may help to reduce the likelihood and severity of post-cardiac arrest syndrome. A more complete coagulation and platelet analysis in larger animal models is required to establish if ALM may be clinically useful.

8.3 General conclusions

Cardiac rescue and haemodynamic stability are essential functions post-cardiac arrest *before* neurological complication can occur (El-Menyar, 2005; Mongardon*, et al.*, 2011; Nolan*, et al.*, 2008). No drug therapy with high quality CPR, with or without shocks, has demonstrated a survival benefit following cardiac arrest in humans (Olasveengen*, et al.*, 2009b; Papastylianou & Mentzelopoulos, 2012; Sunde & Olasveengen, 2014).

This thesis showed that a bolus of AL or ALM immediately prior to chest compressions offers numerous benefits after asphyxial-induced cardiac arrest in rats that might be relevant to clinical settings:

- AL may pharmacologically condition the heart by making it more compliant with higher developed arterial pressures. This finding, along with constant mechanical chest compression devices, may find clinical utility and buy more time for paramedics and first responders on location or during pre-hospital transport (ambulance or air) to definitive care (Fox*, et al.*, 2013).
- AL in conjunction with induced hypothermia starting before resuscitation could improve ROSC attainment during interrupted chest compressions. This benefit is possibly important in clinical practice considering CPR interruptions are often inevitable in out-of-hospital, during transport and in-hospital settings (Abella*, et al.*, 2005a; Olasveengen*, et al.*, 2008; Wik*, et al.*, 2005), which directly lead to worse outcomes (Souchtchenko*, et al.*, 2013).
- AL or ALM treatment may prevent fatal arrhythmias (VF) during or after chest compressions. This could be imperative for survival since ~70% OHCA patients experience recurrent VF during chest compressions (Berdowski*, et al.*, 2010). In addition, the anti-arrhythmic properties of AL (and ALM) are vigorous even during moderate hypothermia. This may find a clinical relevance since overcooling (<32^{o}C) often occurs in cardiac arrest patients (Gillies*, et al.*, 2010; Merchant*, et al.*, 2006), and is associated with increased risk of ventricular arrhythmias (Polderman & Herold, 2009).
- ALM treatment together with intra-arrest hypothermia was superior compared to other treatments to generate ROSC, improve haemodynamics, stabilise the ECG and prevent coagulopathy post-resuscitation. It may offer clinical advantages to improve haemodynamic instability and coagulopathy post-resuscitation in cardiac arrest patients (Adrie*, et al.*, 2004; Adrie*, et al.*, 2005; Laurent*, et al.*, 2002).

The work in this thesis may also have applications for VF-induced cardiac arrest states, as recent studies have shown that elective compressions can improve the restoration of circulation *before* any attempt is made to defibrillate (Chamberlain*, et al.*, 2008). Moreover, the model employed in this thesis does mimic the clinical scenario in infants and children, as asphyxia from apnoea or progressive hyopoxaemia or hypoventilation are the principle causes of cardiovascular collapse in young patients (Fink*, et al.*, 2004). This may offer a new therapy for near-drowning victims, choking, respiratory diseases and hangings.

8.4 Limitation and future studies

The animal model applied in this thesis has several limitations. The 8 min asphyxial cardiac arrest resulted in four to five minutes circulatory arrest, while out-of-hospital cardiac arrest often proceed for more than 10 minutes before resuscitation is first attempted (Rittenberger*, et al.*, 2006). The brief arrest period may be more relevant to in-hospital or witnessed cardiac arrest, which frequently have shorter resuscitation time delays (Fredriksson*, et al.*, 2010; Hostler*, et al.*, 2010). In addition, extrapolating the experimental results from the rat model to humans could be challenging due to cardiovascular disparities between the two species. These include heart anatomic and electrophysiology features, heart rate and energy metabolic rate (Anderson*, et al.*, 2006; Dobson & Himmelreich, 2002). Moreover, the model was applied in healthy rats, whereas most cardiac arrest patients have underlying cardiac and respiratory diseases or other comorbidities preceding the event.

Potential future studies include translating the findings from the rat to a pig model of asphyxial hypoxia and include more detailed *in vivo* cardiac and brain rescue, inflammatory and coagulation analysis. ALM in physiological (0.9%) saline could be compared to hypertonic (3%) saline ALM, which has been investigated in haemorrhagic shock models in rats and pigs with good outcomes (Granfeldt*, et al.*, 2014; Letson*, et al.*, 2012). In addition, further studies are required to compare ALM with standard-of-care epinephrine, vasopressin and/or amiodarone bolus treatments, with and without different levels of therapeutic pre-and post-ROSC hypothermia, and with łextended 48-72 hour recovery times. Another important area of investigation is the effect of AL or ALM on neural function, since the present study in Chapter 6 was limited by the short arrest and resuscitation periods and brain sampling. Longer monitoring times, immunohistochemical analysis and MRI would be of interest.

A particular focus in future studies would be to investigate AL or ALM effects in near-drowning victims or perinatal asphyxia. The World Health Organization estimates 359,000 persons around the world die from drowning each year, and it is the third cause of unintentional injury death in children ages 5–14 years (Topjian*, et al.*, 2012). Asphyxial hypoxia is particularly common in paediatric and neonatal intensive care units (Topjian*, et al.*, 2012; Tress*, et al.*, 2010), and perinatal asphyxia contributes to around one million newborn deaths annually (Lawn*, et al.*, 2006). A bolus of AL or ALM may offer therapeutical benefits for these conditions and, given the results presented in this thesis, warrants further exploration.

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