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Plasma Endothelin-1 Level at the Onset of Ischemic Ventricular Fibrillation Predicts Resuscitation Outcome

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Abstract

Background—Endogenous vasopressors, including endothelin-1 (ET-1), have been shown to be elevated in patients following resuscitation from out-of-hospital cardiac arrest and are likely a physiologic response to global ischaemia. The importance of ET-1 in the setting of arrest and resuscitation has not been established. Prior work has demonstrated that ET-1 increases significantly after coronary occlusion. The purpose of this study was to assess changes in ET-1 following induction of ischaemia and VF.

Methods—VF was induced in 30 anesthetized and instrumented swine by balloon occlusion of the LAD. Blood was collected from the right atrium at baseline and at 5 minute intervals following LAD occlusion until VF occurred. After 7 min of VF, resuscitation was attempted in accordance with guidelines. ET-1 and matrix metalloproteinase-9 (MMP-9), a measure of infarct size, were measured using ELISA.

Results—ET-1 and MMP-9 levels increased significantly from baseline within 20 min of occlusion of the LAD. Animals that could not be resuscitated had a higher ET-1 ($p = 0.031$) at VF onset but similar ischaemia time (time to VF) and MMP-9, reflecting infarct size. An ET-1 level > 4 pg/ml had a likelihood ratio of 4 for predicting resuscitation failure.

Conclusions—Elevated levels of ET-1 during acute ischaemia predict resuscitation failure independent of the time to VF. This finding may be due to the known effect of ET-1 on coronary vascular resistance or ventricular compliance, resulting in early ischemic contracture.

Keywords

cardiac arrest; ventricular fibrillation; endothelin; resuscitation

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Conflicts of Interest All the authors declare that there are no financial and personal relationships with other people or organisations that could inappropriately influence or bias their work.

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INTRODUCTION

Endothelins (ET) are 21-amino-acid peptides produced by the endothelium and play an important role in cardiovascular physiology during acute myocardial ischaemia. ET-1 reacts with membrane G-proteins resulting in peripheral vasoconstriction and increased inotropy and chronotropy.¹ Vasoconstriction is mediated via a non-adrenergic mediated pathway resulting in an increase in intracellular calcium in vascular smooth muscle cells.^{1,2} ET-1 increases during coronary occlusion and may play a role in arrhythmogenesis and myocardial dysfunction, as well as clinical outcomes.^{3,4}

Plasma ET-1 has been shown to increase in septic, haemorrhagic, and cardiogenic shock.⁵ It has been suggested that the endothelium, functioning as an autocrine and paracrine organ, is an important mediator of responses to cardiac arrest, resuscitation, and reperfusion.⁶ ET-1 changes during cardiac arrest or following resuscitation have not been well characterized. Two clinical studies suggest that endothelin levels, measured using radioimmunoassay methods, are not elevated following cardiac arrest and resuscitation.^{7,8} A decline in ET-1 levels after resuscitation has been observed in non-survivors when compared to survivors and a positive association between ET-1 and outcome has been reported.⁷ However, the varying sampling intervals and the administration of catecholamines may have effected the observed temporal changes.

The purpose of this study was to investigate the relationship between ET-1 levels and successful resuscitation in a porcine model of ischemically induced ventricular fibrillation and resuscitation. The laboratory model allows more precise sampling intervals and measurement of variables of interest before arrest and resuscitation. Our null hypothesis was that ET-1 would not be different in surviving and non-surviving animals.

METHODS

This investigation was approved by the Animal Care and Utilization Review Committee of our institution and adheres to the American Physiological Society's Guiding Principles in the Care and Use of Animals.

Male mixed breed Yorkshire swine (n =30, mean weight 41 ± 5 kg) were premedicated with ketamine (20 mg/kg) and xylazine (2 mg/kg). General anaesthesia was induced with isoflurane via nose cone and, following endotracheal intubation, maintained with inhaled isoflurane (MAC 1.0-2.5%) and nitrous oxide in a 1 to 1 mixture with oxygen. End-tidal CO₂ was continuously monitored and minute ventilation was adjusted to maintain end-tidal CO₂ at 35-45 mm Hg. Standard lead II of the surface ECG was monitored continuously during instrumentation and throughout the study protocol.

Under fluoroscopic guidance, high fidelity, micro-manometer tipped catheters (Millar Instruments, Houston, TX) were positioned in the ascending aorta via a femoral artery and in the right atrium (RA) via a jugular vein. Commercially available, standard adhesive defibrillation electrode patches (Quick-Combo, Medtronic Emergency Response Systems, Redmond, WA) with an active surface area of approximately 115 cm² were applied to the left and right lateral aspects of the shaved thorax. Transthoracic impedance was measured using a tetrapolar constant current impedance measuring system (THRIM®, Morro Bay, CA). A small value non-inductive resistor (30Ω) was then placed in series with the truncated exponential biphasic defibrillation waveform defibrillator (LifePak 12, Medtronic Emergency Response Systems, Redmond, WA).

Following instrumentation, heart rate, systolic and diastolic aortic pressure and mean RA pressure, were recorded and arterial blood was analyzed (I-Stat EG7+, I-Stat Corp, Princeton,

NJ). Using a 6 French guiding catheter inserted via a carotid artery, a 4.0mm × 20mm Voyager angioplasty catheter (Abbott Vascular, Temecula CA) was positioned over a standard 0.014 coronary wire in the left anterior descending (LAD) coronary artery distal to the first septal perforator. The balloon was then inflated to 6-8 atmospheres. The site of coronary occlusion and confirmation of complete cessation of coronary flow distal to the balloon were confirmed with manual contrast injections.

Seven minutes after the occurrence of spontaneous, ischaemic VF, closed-chest compressions were begun with a mechanical device (Thumper, Michigan Instruments, Grand Rapids, MI) at a rate of 100 compressions/min with force sufficient to depress the sternum 1.5 to 2.0 inches. The occluding balloon remained inflated throughout resuscitative efforts. One minute after starting chest compressions, a transthoracic countershock at 200 J was given. For the purpose of these experiments, successful defibrillation was defined as termination of VF, regardless of the postshock cardiac rhythm or haemodynamic outcome, e.g., spontaneous QRS complexes with or without associated arterial pressure pulses, determined 5 sec after a defibrillation shock.⁹ If VF persisted, additional shocks in an escalating energy sequence (300, 360J) were administered. Chest compressions were performed between shocks and positive pressure ventilations ($F_iO_2=1.00$) were performed at a rate of 8-10 ventilations/minute. If VF persisted after the initial three shocks, epinephrine, 0.5 mg (approximately 0.01 mg/kg), was administered and CPR continued for one to three minutes before additional shocks at 360 J were given. If VF persisted, additional epinephrine at doses of 0.5 mg and amiodarone, 150 mg, or lidocaine, 1 mg/kg, were given, CPR continued, and shocks repeated until VF was terminated or for 15 minutes. If asystole or pulseless electrical activity (PEA) followed shocks, CPR and additional epinephrine were administered until spontaneous arterial pressures of 60 mm Hg appeared or for 15 min. At the end of 15 min of CPR, animals remaining in VF, PEA, or asystole were considered resuscitation failures and resuscitative efforts terminated.

In those animals achieving return of spontaneous circulation (ROSC), defined as an arterial systolic pressure >60 mm Hg for >10 min,¹⁰ haemodynamic and blood gas measurements were made at intervals for one hour. The LAD balloon remained inflated during the 60 minute postresuscitation period. If at any time systolic arterial pressure fell below 50 mm Hg for >10 min, dopamine was administered as a constant infusion and titrated to maintain a systolic arterial pressure of >90 mm Hg. Haemodynamic data were recorded and stored on a lap-top computer using PowerLab Chart v. 5.2 (ADInstruments, Colorado Springs, CO).

Prior to LAD balloon inflation and at 5 minute intervals until VF occurrence, blood was sampled from the right atrium, placed in sterile, chilled (0° C), EDTA coated tubes, and centrifuged at 5000 rpm for 10 min. Plasma was immediately separated and stored at -80° C until analysis. Endothelin-1 (ET-1) and matrix metalloproteinase-9 (MMP-9) concentrations were determined by a quantitative sandwich ELISA using commercially available kits specific for these porcine cytokines (R&D Systems, Inc., Minneapolis, MN). MMP-9 was selected as a marker of acute ischaemia and infarct size.¹¹

Data are presented as the mean ± SD unless otherwise stated. For all comparisons, $p < 0.05$ was considered statistically significant. ET-1 and MMP-9 concentrations at the selected time intervals were compared using the student t-test. Changes over time were assessed using Friedman repeated measures ANOVA on ranks and Dunnett's test. CPR coronary perfusion pressure (diastolic or relaxation phase aortic minus right atrial pressure) was determined by measuring the mean peak CPP over the 30 sec period (approximately 50 compression cycles) preceding the first countershock.

RESULTS

Seventeen animals were successfully resuscitated (Group 1) and 13 were not (Group 2). Time to occurrence of spontaneous VF after occlusion of the LAD was not significantly different between groups (group 1, 1451 ± 429 sec; group 2, 1400 ± 495 sec, $p = 0.766$). Transthoracic impedance for the two groups was not statistically different (group 1, 70 ± 3 ohms; group 2, 68 ± 3 ohms, $p = 0.164$) nor was CPR CPP (group 1, 12 ± 4 mm Hg; group 2, 12 ± 7 mm Hg, $p = 0.813$).

Changes in ET-1 and MMP-9 levels for the two groups are shown in Figures 1 and 2. ET-1 and MMP-9 increased significantly from prearrest values in both groups over time. However, differences in mean values between groups were not apparent at any time point. A significantly greater ($p = 0.031$) peak ET-1 level, determined at the time point preceding VF, was observed in non-resuscitated animals (3.34 ± 1.75 pg/mg) when compared to those animals that achieved restoration of spontaneous circulation (2.00 ± 1.48 pg/ml). An ET-1 level > 4 pg/ml at the onset of VF had a positive predictive value of 75% and a likelihood ratio of 4 for predicting resuscitation failure (Figure 3).

There was no correlation (Spearman Rank Order Correlation) between the peak ET-1 level and time to VF (correlation coefficient = -0.087 ; p value = 0.645)

DISCUSSION

Elevated acute phase ET-1 levels portend a poor prognosis in patients with acute myocardial infarction due to an increased incidence of ventricular tachycardia, lowered systolic ejection fraction, cardiogenic shock, and cardiac death.¹² Our study demonstrates that ET-1 levels increase significantly within 20 minutes of coronary occlusion and that the peak levels of ET-1 are related to the likelihood of successful resuscitation. Higher levels of ET-1 are known to reduce regional myocardial perfusion, due to the effects of ET-1 on coronary vascular resistance, and may contribute to early ischaemic contracture by increasing cytosolic calcium concentration.

ET-1 mediates vasoconstriction via a non-adrenergic-mediated increase in calcium concentration within vascular smooth muscle cells.^{1,2} Administration of exogenous ET-1 has been shown to increase coronary perfusion pressures in the porcine arrest-resuscitation model, but may worsen resuscitation outcome.¹³⁻¹⁵ Although coronary perfusion pressure increases following ET-1, the increase in coronary vascular resistance may preclude an increase in actual coronary blood flow. Alternatively, the increase in resistance may decrease flow, decreasing the likelihood of successful resuscitation and contribute to post-resuscitation myocardial dysfunction.

ET-1 levels and the density of ET-1 receptors in the left ventricle have been shown to increase during myocardial ischaemia and are associated with increased expression of ET-1 messenger RNA in the porcine ischaemia model.¹⁶ Decreased levels of ET-1 in the porcine ischaemia model have been associated with decreased myocardial infarct size.¹⁷ Activation of endothelin receptors has been shown to result in ischaemic contracture via activation of sodium-hydrogen exchange and an increase in cytosolic calcium. Contracture can be prevented or attenuated by either ET-1 blockers or calcium channel entry blockers.¹⁸

To our knowledge, only two studies have evaluated the potential role of endogenous ET-1 in the setting of cardiac arrest and resuscitation. Haynes and colleagues demonstrated that initial plasma endothelin-1 levels following resuscitation were not different from those measured in a normal control population.⁷ Similarly, initial levels were not different between patients who eventually survived and those who did not. However, endothelin levels decreased over time in

the nonsurvivors. The investigators concluded that the potent vasoconstrictor effects of ET-1 may be beneficial in the early post-resuscitation period. Lindner and coworkers measured ET-1 and other endothelins during arrest and resuscitation in patients who experienced out-of-hospital cardiac arrest.⁸ No differences were observed between survivors and nonsurvivors over time. The findings of Haynes are different than those observed in our study. Differences may be due to two factors. Firstly, we measured the levels of ET-1 prior to arrest, not after resuscitation. Secondly, cardiac arrest was exclusively due to acute cardiac ischaemia in our study animals, while ischaemia as well as other causes of arrest were included in the Haynes' study. Cardiac arrest secondary to a non-ischemic etiology may involve different mechanisms and may explain why increased ET-1 levels were associated with improved outcomes.

There are several limitations to our study. Right atrial samples may not reflect coronary or myocardial tissue levels of ET-1. Due to the anatomy of the porcine coronary sinus, which receives venous drainage from intrathoracic sites other than the heart, coronary sinus samples are unlikely to have yielded more accurate assessment of myocardial ET-1. Regional myocardial perfusion was not measured to more closely quantify the area at risk following occlusion. Moderate differences in infarct size may have influenced resuscitation outcome independent of ET-1 level. However, the site of LAD occlusion, determined angiographically, was approximately the same in all animals (just distal the first septal perforator) and MMP-9 levels were not different between groups following occlusion and before VF onset.^{19,20} Regional flow measurements during CPR may have been helpful to determine if ET-1 increased coronary vascular resistance, thereby yielding lower flow for a given coronary perfusion pressure. Lastly, ET-1 levels in resuscitated animals were not measured during the early post-resuscitation period. Further increases after resuscitation might worsen post-resuscitation ventricular function due to the vasopressor properties of endogenous ET-1.

This study demonstrates that ET-1 levels increase significantly shortly after coronary occlusion and that the peak ET-1 level prior to onset of spontaneous VF is predictive of resuscitation failure using conventional interventions. Induction of important mediators follows coronary occlusion and ischaemia and endothelial "events" preceding VF onset may play a role in eventual resuscitation outcome.

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Figure 1. Plasma Endothelin-1 Concentrations After LAD Occlusion

Plasma endothelin-1 was measured prior to coronary occlusion (time = 0) and at 5 min intervals until spontaneous ventricular fibrillation occurred. Differences were not noted between groups at any time point. ET-1 was significantly greater ($p < 0.05$) than baseline values at the 20, 25, and 30 min time points.

Figure 2. Plasma MMP-9 Concentrations After LAD Occlusion

Plasma MMP-9 was measured prior to coronary occlusion (time = 0) and at 5 min intervals until spontaneous ventricular fibrillation occurred. Differences were not noted between groups at any time point. MMP-9 was significantly greater ($p < 0.05$) than baseline values at the 20, 25, and 30 min time points.

Figure 3. Endothelin-1 Level versus Resuscitation Outcome

Peak ET-1 concentration preceding spontaneous VF and outcome are plotted for each study animal. A concentration of >4 pg/ml yielded a likelihood ratio of 4 for resuscitation failure.