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High Serum Tumor Necrosis Factor Levels in the Early Post-Cardiac Arrest Period Are Associated with Poor Short-Term Survival in a Swine Model of Ventricular Fibrillation

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Most resuscitated victims of out-of-hospital cardiac arrest who survive to hospital expire due to the post-resuscitation syndrome. This syndrome is characterized by a sepsis-like proinflammatory state. The objective of this investigation was to determine whether a relationship exists between the rise of tumor necrosis factor (TNF), a proinflammatory cytokine, following return of spontaneous circulation (ROSC), and early postarrest survival in a clinically relevant animal model of spontaneous ventricular fibrillation (VF). Mixed-breed Yorkshire swine ($n = 20$), weighing 39 ± 5 kg, were anesthetized and catheters placed in the right atrium and left ventricle/ascending aorta for continuous pressure monitoring. VF was induced by occluding the left anterior descending coronary artery with an angioplasty balloon. After 7 min of untreated VF, advanced life support resuscitation attempts were made for up to 20 min. Animals achieving ROSC were monitored for 3 h and fluid and pressor support was administered as needed. TNF levels were measured before VF and at 0, 15, and 30 min after ROSC using quantitative sandwich enzyme-linked immunosorbent assay. Twelve (60%) animals experienced early death, expiring during the 3 hour postarrest period (9 pulseless electrical activity, 2 VF, and 1 asystole). The TNF level at 15 min post-ROSC was significantly associated with death within the first 3 h post-ROSC with a univariate odds ratio of 1.4 [95% confidence interval (CI) 1.05–2.2, $P = 0.01$]. Using a cutoff TNF level of 525 pg/mL at 15 min post-ROSC had 100% negative predictive value (95% CI 0%–37%) and 67% positive predictive value (95% CI 35%–90%) for early death with a hazard ratio of 6.6 (95% CI 1.9–23.5). TNF increases shortly after ROSC and is predictive of early death. Early identification of resuscitated victims at greatest risk for hemodynamic collapse and recurrent arrest might facilitate the use of early hospital-based interventions to decrease the likelihood of a poor outcome.

Keywords: ventricular fibrillation, cardiac arrest, post-cardiac arrest care, cytokines

Introduction

Approximately 300,000 people in the United States experience out-of-hospital cardiac arrest annually. Resuscitation efforts are unsuccessful in most and only about 25% survive to hospital admission and 10% survive to hospital discharge (Stub and others 2011). The high in-hospital mortality rate has been ascribed to the post-resuscitation syndrome, which is multifactorial in origin (Neumar and others 2008). A systemic response to ischemia and reperfusion plays an important role in this syndrome and is manifested by a ''sepsis-like'' inflammatory state during the early postresuscitation period (Adrie and others 2002; Neumar and others 2008). The proinflammatory cytokines, particularly tumor necrosis factor (TNF), have been implicated in myocardial dysfunction, neuronal injury, and death following resuscitation in animal models (Niemann and others 2009; Janata and others 2014).

An increase in plasma cytokines has been demonstrated early during resuscitation and after return of spontaneous circulation (ROSC) and is associated with a decline in myocardial contractility (Niemann and others 2009). Early identification of resuscitated patients at greatest risk for hemodynamic collapse and recurrent arrest might facilitate early intervention with pressor support, early percutaneous coronary intervention, or extracorporeal circulation and decrease the likelihood of postresuscitation death due to irreversible cardiac or multiorgan failure.

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Swine are now the most commonly used species in resuscitation research, largely because swine coronary anatomy and collateral circulation closely resemble that in humans, as does the physiologic response to ischemia/reperfusion.

Objectives

The objective of this study was to determine the relationship between the early TNF response to ischemia and reperfusion and early postarrest survival in a clinically relevant animal model of spontaneous ventricular fibrillation (VF).

Materials and Methods

Ethical statement

This experiment conforms to standards for the ethical treatment of laboratory animals and was approved by the local Institutional Animal Care and Use Committee at LABioMed in Torrance, CA (Institutional Protocol 0114220400).

Housing and husbandry

The Animal Care Facility at LABiomed is AAALAC accredited, OLAW assured, and USDA registered. Swine were group caged outdoors for socialization and cages contained balls and tires for environmental enrichment. Animals had free access to water and standard swine chow until the midnight before the experiment when they were allowed water only. Other than periodic observation, there were no specific welfare-related assessments carried out before experiments.

Study design

This report represents a secondary analysis of observational laboratory data obtained during the course of experiments in a study of post-cardiac arrest TNF blockade for the treatment of myocardial dysfunction. The animals in this report come from 20 animals randomized to the control group.

Experimental animals and procedures

The experimental protocol has been described previously and was performed in the morning or afternoon hours between 8 AM and 5 PM (Niemann and others 2007). Briefly, mixed-breed Yorkshire swine ($n = 20$), purchased from a commercial breeder and weighing 39 ± 5 kg, which had been fasted overnight with *ad libitum* access to water before the experiment, were anesthetized with intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg) and isoflurane through nosecone before endotracheal intubation. These agents were selected for their short duration of effect and minimal cardiovascular impact. Animals were placed in a supine position and a general plane of anesthesia was maintained with inhaled isoflurane and nitrous oxide in a 1:1 mixture. This anesthetic mixture was selected for its ease of administration, safety profile, and its minimal myocardial depressant/hemodynamic effects. An $ETCO_2$ of 35–45 mm Hg, measured by sidestream capnometry, was maintained during instrumentation and PaO_2 monitored by arterial blood gas analysis. Lead II of the surface ECG was also monitored throughout the experiment.

Under fluoroscopic guidance, micromanometer-tipped catheters (Milar Instruments, Houston, TX) were placed in the right atrium (RA), ascending aorta (Ao), and left ventricle (LV) by surgical cutdown. Normal saline was administered through the RA catheter at a TKO rate. A pulmonary artery (PA) catheter (Edwards LifeSciences, Irvine, CA) was placed in the left PA for the measurement of cardiac output by thermodilution methods.

Adhesive defibrillation electrode patches were placed on the bilateral shaved thorax. A small-value noninductive resistor (30 Ω) was placed in series with a biphasic defibrillator (LifePak 12; Physio-Control, Redmond, WA). Continuous measurements of Ao, LV, and RA pressures, as well as Lead II, were recorded on a laptop computer (Power Lab Chart v. 5.2; ADInstruments, Colorado Springs, CO).

Focal myocardial ischemia was achieved by inserting a 4 mm × 20 mm angioplasty catheter (Abbott Vascular, Temecula, CA) over a standard 0.014 coronary wire in the left anterior descending coronary artery distal to the first septal perforator through a 6 Fr guidance catheter inserted in a carotid artery. The angioplasty balloon was inflated to 6–8 atms and manual contrast injections were performed to confirm the site of coronary occlusion and complete cessation of coronary flow distal to the balloon.

Animals were then observed until the occurrence of spontaneous VF. After 7 min of untreated VF, manual chest compressions were initiated at a rate of ~100/min with force sufficient to depress the sternum 1.5–2.0 inches (determined visually). The occluding balloon remained inflated throughout resuscitative efforts. One minute after starting chest compressions, transthoracic defibrillation was attempted at 200 J. For the purpose of these experiments, successful defibrillation was defined as termination of VF, regardless of the postshock rhythm or hemodynamic outcome, for example, spontaneous QRS complexes with or without associated arterial pressure pulses, determined 5 s after a defibrillation shock. Additional shocks in an escalating energy sequence (300, 360 J), interposed with chest compressions, were administered if VF persisted. Positive pressure ventilations ($F_iO_2 = 1.00$) were performed at a rate of 8 ventilations/min after the first shock. For animals remaining in arrest, adrenaline, 1 mg, was administered every 3–5 min and cardiopulmonary resuscitation (CPR) was continued with additional shocks at 360 J and amiodarone 150 mg was given as needed for recurrent or refractory VF.

ROSC was defined, for the purposes of this experiment, as an arterial systolic blood pressure (SBP) of at least 60 mm Hg for >2 min. Animals achieving ROSC immediately received 250 mL of normal saline (NS) infused through the RA catheter over 30 min. If the SBP fell below 60 mm Hg for >10 min in the post-ROSC period, a dopamine infusion was initiated and titrated to maintain an SBP >90 mm Hg. The angioplasty balloon remained inflated for the first 60 min of the post-ROSC period and then deflated and removed.

Experimental outcomes

Death during the post-ROSC period and during treatment was defined as the occurrence of refractory hypotension with a systolic arterial pressure <50 mm Hg sustained for >10 min, despite an additional dose of adrenaline and dopamine infusion at a rate >20 μg/kg/min or development of asystole. In stable animals, hemodynamic and blood gas measurements were made at intervals for 3 h.

Arterial whole blood was sampled from the Ao catheter before induction of VF and at 0, 15, and 30 min after ROSC. Samples were immediately placed in sterile, chilled (0°C), ethylenediaminetetraaceticacid-coated tubes, centrifuged at

5,000 rpm for 10 min, and plasma separated and stored at −80°C until batch analysis. TNF levels were determined using quantitative sandwich enzyme-linked immunosorbent assay that is sensitive and specific for porcine TNF from commercially available kits (R&D Systems, Inc., Minneapolis, MN).

Statistical methods

Data were entered into an Excel Spreadsheet (v. 12.0; Microsoft Corp, Redmond, WA) and imported into STATA statistical software (STATA/IC 12.1; StataCorp, College Station, TX) for analysis. A Kaplan-Meier survival plot was used to model survival in the post-cardiac arrest period. Spearman rank correlation was used to assess associations between TNF and variables of interest. Exact logistic regression and Cox proportional hazard models were used to assess the association between TNF levels measured at 15 min postarrest and death within the first 3 h. While 30 min values were also measured, values were missing for animals that expired before this time point. Due to known genetic variability in the cytokine response to ischemia, we defined TNF nonresponders as those animals exhibiting a less than 2× rise in TNF at 15 min when compared to individual control values. We performed separate analysis after excluding these animals. We considered $P > 0.05$ to be statistically significant. All P-values are 2-sided.

Results

Baseline data

Prearrest variables are given in Table 1. Table 2 summarizes resuscitation variables. There was no association between TNF levels at 15 min postarrest and time to ROSC (Spearman's rho = –0.22, $P = 0.36$), nor was time to ROSC associated with death in the first 3 h postarrest. There was also no association between total countershocks, refibrillation, and TNF levels at 15 min.

Outcomes and estimation

Data from all 20/20 (100%) swine were used in the analysis. Twelve (60%) animals experienced early death, expiring during the 3 h postarrest period (9 pulseless electrical activity, 2 VF, and 1 asystole). Table 3 provides TNF levels for all animals at each time point. The Kaplan-Meier survival curve is given in Fig. 1. The TNF level at 15 min post-ROSC was significantly associated with death within the first 3 h with a univariate odds ratio (OR) of 1.4 [95% confidence interval (CI) 1.05–2.2, $P = 0.01$] and a hazard ratio of 1.1 (95% CI 1.02–1.2, $P = 0.01$). Among 8 animals with a 15 min TNF level <525 pg/mL, none died, while 8/12 animals with a 15 min TNF level ≥525 pg/mL died in the first 3 h postarrest. This cutoff had 100% (95% CI 0%–37%) negative predictive value and 67% (95% CI 35%–90%) positive predictive value for early death. In time-to-event analysis, the hazard ratio for animals with a 15 min TNF level ≥525 pg/mL was 6.6 (95% CI 1.9–23.5, Fig. 2).

When we excluded TNF nonresponders (rise in TNF at 30 min postarrest <2× control values), the OR for the association between 15 min TNF and early death was similar: 1.5 (CI 1.02–3.0, $P = 0.03$).

Table 1. Prearrest Variables Among Twenty Swine Before Induction of Ischemically Induced Ventricular Fibrillation

Variable		
Weight (kg)	39	±5
Heart rate (beats/min)	102	±14
Mean arterial pressure (mm Hg)	95	±9
Stroke work (g-m)	45	±11
TNF levels (pg/mL)	68.5	(54–114.5)

Values given as mean ± SD or median (Interquartile Range [IQR]).
SD, standard deviation; TNF, tumor necrosis factor.

Table 2. Resuscitation Variables for Twenty Swine Resuscitated from Ischemically Induced Ventricular Fibrillation

Variable		
Time to ventricular fibrillation (s)	1,374	±367
Milligrams of epinephrine	2	±0.7
Total countershocks	14	(10–16)
Time to ROSC (s)	445	±231

Values given as mean ± SD or median (IQR).
ROSC, return of spontaneous circulation.

Discussion

The proinflammatory cytokine response resulting from the ischemia and reperfusion associated with cardiac arrest and resuscitation has been implicated in the postresuscitation syndrome and the likelihood of survival. In this study, we have demonstrated that the TNF response following onset of arrest, during resuscitation, and shortly after restoration of spontaneous circulation is predictive of early death and short-term survival. Higher levels of TNF were associated with rearrest due to recurrent VF.

Table 3. Tumor Necrosis Factor Levels Among Twenty Swine Resuscitated from Ischemically Induced Ventricular Fibrillation

Animal	*Control TNF (pg/mL)*	*15 min TNF (pg/mL)*	*30 min TNF (pg/mL)*
1	134	1,636	
2	330	1,711	
3	91	1,353	1,377
4	87	520	556
5	58	143	100
6	116	142	129
7	45	731	934
8	66	1,725	
9	73	59	94
10	59	33	56
11	19	107	102
12	56	458	
13	64	318	229
14	21	1,963	1,891
15	71	82	64
16	113	230	217
17	24	35	
18	326	733	876
19	190	190	339
20	52	529	598

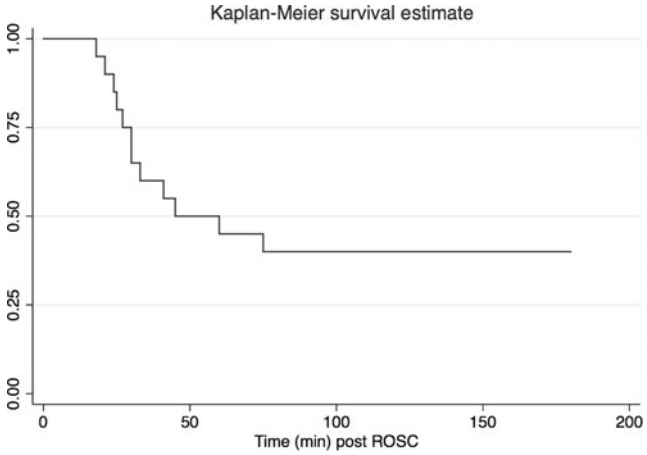

FIG. 1. Kaplan-Meier short-term survival estimate for 20 swine that achieved ROSC following resuscitation from ischemically- induced ventricular fibrillation. ROSC, return of spontaneous circulation.

In both laboratory models and the clinical population, increased blood levels of proinflammatory cytokines, particularly TNF, interleukin (IL)-1β, and IL-6, are noted within hours to days following resuscitation (Adrie and others 2002; Niemann and others 2009; Samborska-Sablik and others 2011). In resuscitated out-of-hospital cardiac patients, the highest levels are observed in patients who die in the hospital following initial resuscitation. In animal cardiac arrest models, a similar increase in TNF has been observed in the central nervous system and is associated with histologic neuronal death (Janata and others 2014). Preliminary observations suggest that inhibiting TNF production or attenuating its effects through receptor blockade may lessen the severity of some components of the post-resuscitation syndrome (Niemann and others 2013; Drabek and others 2014; Gao and others 2015).

The ischemically induced, spontaneous VF cardiac arrest model used in this study differs substantially from the more commonly used electrically induced porcine VF model, particularly with respect to the proinflammatory cytokine response observed after resuscitation (Niemann and others 2008). In swine, plasma TNF concentrations do not change significantly after resuscitation from electrically induced VF. After resuscitation from ischemically induced VF, there is an immediate increase in TNF, which persists after ROSC. This was confirmed in this study. Intra-arrest coronary perfusion pressure and time to ROSC were not significantly different in early survivor and nonsurvivor groups. Early death was typically due to either pressor resistant arterial hypotension or postcountershock pulseless electrically activity following countershock of recurrent VF that failed to respond to chest compressions and epinephrine. Similar hemodynamic instability and recurrent VF have been described in humans and often presage early death (Kilgannon and others 2008; Lin and others 2010; Kaji and others 2011).

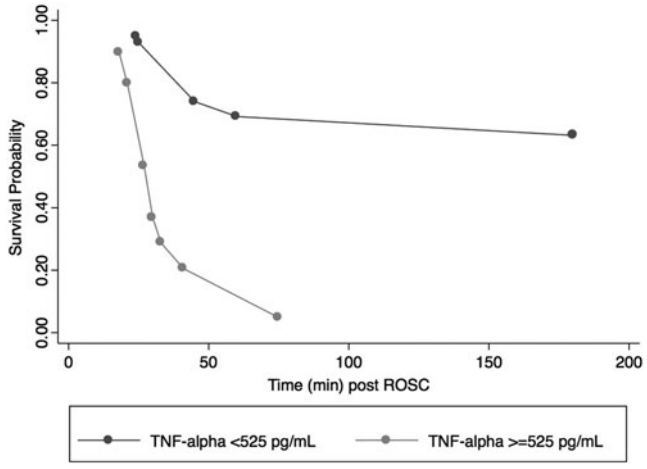

FIG. 2. Cox proportional hazards model comparing swine with a 15 min post-ROSC TNF level of ≥525 pg/mL with a TNF level of <525 pg/mL following resuscitation from ischemically induced ventricular fibrillation. TNF, tumor necrosis factor.

Myocardial TNF mRNA and peptide increase significantly 10 min after induction of myocardial ischemia and peak at 30 min to 3 h (Shames and others 2002; Xiao and others 2008). Although not measured in this study, it is likely that similar changes occurred in the occluded swine myocardium. TNF has previously been demonstrated to play a role in arrhythmogenesis in the setting of experimental acute myocardial infarction (Xiao and others 2008; Chen and others 2011). When added to the perfusate of isolated heart preparations, TNF has been shown to induce ventricular arrhythmias, including VF (Xiao and others 2008). Facilitation of ventricular arrhythmias by TNF can be attenuated by blocking TNF with receptor inhibitors (Chen and others 2011). Exposure of myocytes to TNF may decrease outward potassium current and prolong action potential duration (Fernandez-Velasco and others 2007). Lymphotoxin may also alter intracellular calcium handling and thereby contribute to both depressed contractility and enhanced arrhythmogenesis (Duncan and others 2010).

Our study has several limitations. Although the site of coronary occlusion was consistent and confirmed angiographically in all animals, infarct size produced by balloon occlusion may not have been comparable between animals surviving and those dying early. However, the swine heart can be characterized as having end-artery anatomy with minimal collateralization. The area at risk is likely to have been consistent across study animals. Prior work from our laboratory using matrix metalloproteinase-9 as a marker of infarct size demonstrated remarkable consistency in our ischemic VF model (Shah and others 2009). Other cytokines may have contributed to early death, but were not measured. We have previously demonstrated in this animal model that changes in IL-1β and IL-6 are small and widely variable (Niemann and others 2009). It has been suggested that endothelin-1 may play a role in arrhythmogenesis in the setting of myocardial ischemia (Sharif and others 1998; Raschack and others 1998). Endothelin-1 was not measured in this study, but prior work from our laboratory using the ischemic pig model failed to demonstrate a relationship between endothelin-1 and ventricular arrhythmias (Shah and others 2009).

Conclusions

This study demonstrates that the proinflammatory cytokine TNF increases abruptly after ROSC and is predictive of early death. Early identification of patients at risk for early death after resuscitation from cardiac arrest using a biomarker

might facilitate early intervention with newer emerging therapies, for example, cardiopulmonary bypass and extracorporeal circulatory support or hemofiltration.

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Author Disclosure Statement

No competing financial interests exist.

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