Cardiac Function and the Proinflammatory Cytokine Response After Recovery From Cardiac Arrest in Swine

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Increased levels of cytokines have been reported after resuscitation from cardiac arrest. We hypothesized that proinflammatory cytokines, released in response to ischemia/reperfusion, increase following resuscitation and play a role in post-cardiac arrest myocardial dysfunction. Ventricular fibrillation (VF) was induced by coronary occlusion in 20 swine. After 7 min of VF, resuscitation was performed as per guidelines. Plasma levels of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 were measured 15 min after the start of resuscitation in all animals and at intervals of 6 h in resuscitated animals. Intravascular pressures and cardiac output (CO) were also recorded. TNF- α abruptly increased after resuscitation, peaking at 15 min following return of spontaneous circulation, and declined to baseline levels after 3 h. IL-1 β increased more slowly, reaching a maximum 2 h after reperfusion. IL-6 concentrations were not significantly different from control values at any time point. Males demonstrated greater elevations of TNF- α and IL-1 β than females. Stroke work was significantly depressed at all time points with a nadir at 15–30 min after reperfusion, corresponding to the peak TNF- α values. The anti-TNF- α antibody infliximab attenuated the decrease in myocardial function observed 30 min after reperfusion. TNF- α increases during recovery from cardiac arrest are associated with depression of left ventricle (LV) function. The effect of TNF- α can be attenuated by anti-TNF- α antibodies.

Introduction

IT HAS BEEN ESTIMATED that 250,000 people experience out-of-hospital cardiac arrest each year (Eisenberg and Mengert 2001). Although spontaneous circulation is restored in 40%–50%, the majority of patients initially resuscitated will subsequently die before leaving the hospital, resulting in a hospital survival rate of ~5% in major cities nationwide (Nichol and others 2008). In-hospital death is most often the result of multisystem organ failure due, in part, to profound myocardial dysfunction with depressed cardiac output (CO) and recurrent arrhythmias (Ptacin and others 1982; Deantonio and others, 1990; Mullner and others 1998; Kern 2002; Laurent and others 2002). Progressive and devastating brain injury is common and often the cause of death (Madl and Holzer, 2004). This progressive decline in vital organ function has been called “the post-cardiac arrest syndrome” (Neumar and others 2008).

Cardiac arrest and resuscitation can be viewed as a paradigm for global ischemia and reperfusion, exhibiting many of the metabolic responses described in focal *in vivo*

ischemia models or globally ischemic, isolated whole organ preparations (Schaller and Graf, 2004; Buja 2005; Moens and others, 2005; Harukuni and Bhardwaj 2006). Evidence of oxidant injury appears rapidly after resuscitation from cardiac arrest and activation of characteristic metabolic cascades responsible for reperfusion injury and the accompanying inflammatory response is expected (Basu and others 2000; Idris and others 2005).

Ischemia/reperfusion injury has been characterized as a multifactorial antigen-independent inflammatory condition (Boros and Bromberg 2006). Increases in proinflammatory cytokines and soluble receptors have been reported in patients hours after resuscitation from cardiac arrest and detectable plasma levels of endotoxin appear within days, presumably due to gut translocation (Adrie and others 2002). An association between ischemia and reperfusion and the innate inflammatory response has been suggested and eventual nonsurvivors have greater cytokine elevations following resuscitation than survivors.

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Observational studies in small groups of patients resuscitated from cardiac arrest have evaluated nonspecific acute phase response proteins or 1 or more cytokines following the return of circulation in cardiac arrest victims (Shyu and others 1997; Oppert and others 1999; Mussack and others 2001; Mussack and others 2002). However, sampling occurred early and infrequently and the population was heterogeneous with respect to cardiac arrest duration and post-recovery hemodynamic status. Nonetheless, these studies demonstrated the activation of an early inflammatory response following whole body reperfusion after cardiac arrest, as well as participation of the cytokines in this response.

The purpose of this investigation was to define the early proinflammatory cytokine response after resuscitation during extended observation (6 h) following resuscitation from ischemically induced cardiac arrest and resuscitation of prolonged duration in a porcine model.

Materials and 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.

Domestic swine (Yorkshire and Yorkshire/Hampshire crossbreed) 3 to 4 months of age and of both sexes (males = 10, weight 41 ± 4 kg, females = 10, weight 38 ± 5 kg) were premedicated with ketamine (20 mg/kg) and xylazine (2 mg/kg). General anesthesia 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 CO_2 was continuously monitored and minute ventilation was adjusted to maintain end-tidal CO_2 at 35–45 mmHg. Standard lead II of the surface ECG was monitored continuously during instrumentation and throughout the study protocol.

Under fluoroscopic guidance, high-fidelity micromanometer tipped catheters (Millar Instruments, Houston, TX) were positioned in the ascending aorta and left ventricle (LV) via the femoral arteries and in the right atrium (RA) via a jugular vein. A thermistor-tipped catheter (Edwards Lifesciences, Irvine, CA) was positioned in a branch of the pulmonary artery for thermodilution CO determinations. Commercially available, standard adhesive defibrillation electrode patches 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 (30 Ω) 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 and LV pressure, mean RA pressure, LV systolic (max) and diastolic (min) dP/dt , and CO were recorded and arterial blood was analyzed (I-Stat CG8+, I-Stat Corp, Princeton, NJ). Mean arterial pressure (MAP), stroke volume (SV), and LV stroke work were derived using standard formulae. Using a six French guiding catheter inserted via a carotid artery, a 4 mm $\times$ 20 mm 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 atm. The site of coronary occlusion and confirmation of complete cessation of coronary flow distal to the balloon were confirmed with manual contrast injections. Spontaneous ventricular fibrillation (VF) occurred in all animals.

After 7 min of untreated VF, manual chest compressions were begun at a rate of $\sim 100/\text{min}$ with force sufficient to depress the sternum 1.5 to 2.0 in. with the animal in the supine position. The occluding balloon remained inflated throughout resuscitative efforts. One min 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 post-shock cardiac rhythm or hemodynamic outcome, for example, spontaneous QRS complexes with or without associated arterial pressure pulses determined 5 s after a defibrillation shock (Gliner and White 1999). If VF persisted, additional shocks in an escalating energy sequence (300, 360 J) were administered. Chest compressions were performed between shocks and positive pressure ventilations ($\text{F}_i\text{O}_2 = 1.00$) were performed at a rate of 8 ventilations/min. If VF persisted after the initial 3 shocks, epinephrine, 0.5 mg (~ 0.01 mg/kg), was administered and CPR continued for 1 to 3 min 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 min. If asystole or pulseless electrical activity (PEA) followed shocks, CPR and additional epinephrine were administered until spontaneous arterial pressures of 60 mmHg 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 after blood sampling.

In those animals achieving return of spontaneous circulation (ROSC), defined as an arterial systolic pressure >60 mmHg for >10 min (Idris and others 1996), hemodynamic and blood gas measurements were made at intervals for 6 h. The LAD balloon was deflated 60 min after restoration of spontaneous circulation. If at any time systolic arterial pressure fell below 60 mmHg for >10 min, dopamine was administered as a constant infusion and titrated to maintain a systolic arterial pressure of >90 mmHg. Post-resuscitation cardiac function was assessed with measurements of heart rate, arterial, right atrial, and LV pressures, LV systolic and diastolic dP/dt , and thermodilution cardiac output. Hemodynamic data were recorded and stored on a laptop computer using PowerLab Chart v. 5.2 (ADInstruments, Colorado Springs, CO).

Prior to LAD balloon inflation and at 15, 30, 60 min following restoration of circulation and at hourly intervals thereafter, arterial blood was sampled, placed in sterile, chilled (0°C), heparinized tubes, and centrifuged at 5,000 rpm for 10 min. Plasma was immediately separated and stored at -80°C until analysis. $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6 concentrations were determined by a quantitative sandwich ELISA using commercially available kits specific for these porcine cytokines (R&D Systems, Inc., Minneapolis, MN). These assays are specific for porcine $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6 .

Plasma 17-estradiol and testosterone levels were measured in 7 male and 10 female study swine. Estradiol was measured by direct immunoassay (Diagnostic Systems

Laboratories, Webster, TX) and testosterone was measured using a specific radioimmunoassay kit (Diagnostic Products, Los Angeles, CA).

To determine if the anesthetic regimen or surgical procedures used in the experimental group might effect cytokine production, 3 male swine were included as sham controls. These animals were anesthetized and instrumented in the same manner as experimental animals. After a 15-min period representing a "sham" arrest and resuscitation time period, blood was sampled at the intervals noted above and assayed for TNF- α , IL-1 β , and IL-6.

Data were entered into a database and translated to native SAS format using DBMS/Copy Version 8 (Dataflux Corporation, Cary, NC). Values are reported as the mean and standard deviation or the median with interquartile ranges. Control, prearrest hemodynamic data were compared to those recorded during the post-resuscitation period using the SAS procedure PROC MIXED to model mean arterial pressure and LV stroke work. We used a repeated measures, fixed effects linear model for the effect of time since ROSC and a Dunnett-Hsu correction to adjust for the multiple tests associated with comparing each time point to control. A compound symmetry covariance structure was assumed.

The statistical analysis of the cytokine measurements was designed to take several factors into account. First, the data were not expected to be normally distributed, with a substantial number of zero values and a large range. Second, expected correlations of measurements within animals required use of a repeated measures analysis. To account for these issues, values are reported as medians with interquartile ranges (IQRs) and, prior to regression analysis, values were transformed using the log of 1 plus the measured value. Multivariable linear models with repeated measures were implemented using SAS version 9.1 and PROC MIXED. Each time point was compared to the baseline measurements at time zero with a Dunnett-Hsu correction for multiple comparisons. A compound symmetric covariance structure was assumed.

Initially, models were constructed using only time as a categorical predictor (ie, assuming no particular functional dependence of the measurements on time) but it was visually noted that there appeared to be a systematic difference in cytokine concentrations as a function of animal gender. Additional models, using both time and gender as predictors, confirmed this gender effect. Accordingly, the primary regression results, including tests of statistical significance, are based on models that incorporated both time and gender as predictors.

Because the regression analyses were conducted using log-transformed data, the estimates of regression coefficients are expressed as multiplicative factors, relative to baseline cytokine measurements obtained at time zero on female animals. Ninety-five percent confidence intervals (95% CIs) for these multiplicative factors were calculated but were not adjusted for multiple comparisons. *P* values for the statistical significance of these regression coefficients were calculated as well—these tests of statistical significance were adjusted for multiple comparisons to baseline using the Dunnett-Hsu adjustment as mentioned earlier.

Results

Time to VF following coronary occlusion was 1228 ± 804 s and the mean number of countershocks required to

terminate VF was 3 ± 2 . Recurrent VF was common (5 ± 4 episodes per animal) necessitating a total of 11 ± 5 shocks per animal. Time to restoration of spontaneous circulation was 604 ± 129 s. The mean total ischemia time (untreated VF duration and resuscitation time) was 17 min.

Pearrest and post-resuscitation hemodynamic data are shown in Figure 1. Mean arterial pressure and LV stroke work were significantly depressed throughout the post-resuscitation observation period when compared to control (time = 0) values.

Variations in cytokine levels following reperfusion are shown in Figure 2 for the group as a whole. TNF- α and IL-1 β significantly increased within the first 30 min. TNF- α and IL-1 β were not statistically different from prearrest control values when measured at 3 and 4 h, respectively, after return of spontaneous circulation. IL-6 varied over time but showed no significant variation from control values.

To determine if the anesthetic regimen or surgical procedures performed during the course of this study effected concentrations of the measured cytokines, 3 male swine were anesthetized and instrumented in a manner similar to the study group. These animals were not subjected to ventricular fibrillation arrest and resuscitation but did have blood samples obtained at intervals comparable to those of the study group. Significant variations were not observed in these sham control animals (Fig. 3).

When plasma cytokine concentrations were analyzed with respect to swine gender, male swine had significantly higher TNF- α when compared to females. IL-1 β and IL-6 did not vary with gender (Fig. 4).

The results of multivariable, repeated measures linear regression models for cytokine variations over time are shown in Table 1. All regression estimates are expressed as multiplicative factors, relative to cytokine measurements on

FIG. 1. Mean arterial pressure (MAP) and left ventricular stroke work (SW) following reperfusion. Data are presented as the median with interquartile ranges (25%–75%). Significant differences between prearrest (time = 0) and postarrest MAP and SW values were observed at all time points. Numbers above each time point represent the number of animals surviving to that time point.

FIG. 2. Cytokine concentrations following reperfusion. The time course of tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , and IL-6 plasma concentrations after resuscitation are shown. Median cytokine concentrations are plotted with error bars indicating interquartile ranges (IQRs). The statistical significance of values at each time point based on multivariable repeated measures regression when compared with the baseline measurements at time zero is indicated with symbols above the error bars. Time points after control (time = 0) represent sampling times after restoration of circulation.

a female animal at time zero. Ninety-five percent confidence intervals (95% CIs) are shown for each time point and for male gender relative to female gender—these CIs are not corrected for multiple comparisons. The *P* values shown

FIG. 3. Cytokine concentrations of tumor necrosis factor- α (TNF- α), interleukin (IL)-1, and IL-6 in sham controls. These animals were subjected to the anesthetic regimen and surgical procedures of the experimental group but were not subjected to ventricular fibrillation or resuscitation. Cytokine concentrations over 6 h were determined at time intervals identical to those of the treatment group. Significant changes were not observed over the study period.

in the table, however, have been adjusted multiple comparisons to baseline, using the Dunnett-Hsu adjustment in SAS Proc Mixed. The TNF- α results demonstrate a statistically significant elevation from 15 min through 180 min, whereas the IL-1 β values were elevated significantly at all time points. In contrast, IL-6 measurements were not statistically significantly elevated at any time point, although the 95% CIs are quite large because of substantial variability in the measurements.

Testosterone and 17-estradiol levels (median and interquartile range) were not significantly different between genders (male testosterone 4 [3,11] pg/mL, female testosterone 6 [3,7] pg/mL; male estradiol 44 [10,62] pg/mL, female 17 [9–64] pg/mL). Differences in TNF- α concentrations over time were not observed when values were compared between male and female swine with a 17-estradiol level of <20 pg/mL and >20 pg/mL (Fig. 5).

Infliximab study

To determine if TNF- α contributes to post-resuscitation myocardial dysfunction, 6 additional male swine were studied. These animals were instrumented as previously described. However, VF was induced using the conventional method of passing AC current through a bipolar electrode positioned in contact with the right ventricular endocardium. After 7 min of untreated VF, resuscitation efforts were initiated in a manner identical to the methods described for the ischemic study group. Following resuscitation, animals received an infusion of infliximab (5 mg/kg in 250 mL of normal saline administered over 30 min beginning 5 min after restoring spontaneous circulation, *n* = 3) or normal saline alone (*n* = 3). Swine were observed for 3 h and hemodynamic measurements and blood sampling for cytokines were performed at intervals.

FIG. 4. Cytokine levels and gender. The time dependence of tumor necrosis factor- α (TNF- α), interleukin (IL)-1, and IL-6 concentrations after resuscitation are separated by gender. Median cytokine concentrations are plotted with error bars indicating interquartile ranges. Gender differences were determined using multivariable regression modeling, and for TNF- α the gender differences were highly significant ($P < 0.0001$). Gender differences were not statistically significant for IL-1 and IL-6.

Infliximab significantly attenuated the early post-resuscitation decrease in stroke work when compared to saline-treated animals (Fig. 6). However, by 60 min after return of circulation, LV stroke work in the infliximab-treated animals was similar to that of the saline-treated group. Plasma levels

of TNF- α antigen were not significantly different between groups at all times tested (Fig. 7).

Discussion

The immediate reperfusion period after restoration of spontaneous circulation following cardiac arrest is characterized by an abrupt increase in plasma TNF- α concentration followed by elevations in IL-1 β . TNF- α and IL-1 β elevations were transient, with values returning to control prearrest levels within 3 h of reperfusion. Peak TNF- α and IL-1 β levels were observed at the nadir of LV dysfunction. This proinflammatory cytokine response after resuscitation may play a role in post-resuscitation myocardial dysfunction, an important component of the post-resuscitation syndrome. This is supported by our preliminary observations demonstrating that the anti-TNF- α antibody infliximab attenuates the typically observed early decrease in myocardial contractile function. Although an increase in IL-6 values was observed in some males and females during early reperfusion, these changes failed to attain statistical significance. The small changes in IL-6 may indicate that this cytokine does not play an important role in the response to ischemia and reperfusion in this model. Alternatively, the observation period following reperfusion may have been too short to detect what might be an important contribution of this cytokine to eventual outcome.

Ito and colleagues were among the first to suggest that TNF- α may play a role in the post-cardiac arrest syndrome (Ito and others 2001). IL-8 and TNF- α were measured in a heterogeneous group of cardiac arrest survivors. Greater IL-8, but not TNF- α , concentrations were observed in patients who died early or who demonstrated profound neurologic dysfunction within 1 week.

Adrie and coworkers measured plasma cytokines and endotoxin and *ex vivo* cytokine production in patients resuscitated from prolonged cardiac arrest (Adrie and others 2002). TNF- α concentration was elevated at the time of hospital admission (range 0–30 pg/mL) when compared to healthy volunteers (0 pg/mL) and remained elevated at 24 h in eventual nonsurvivors and in those survivors requiring catecholamines for hemodynamic support. However, detectable TNF- α was found within the first 2 days in only about half the patients. Plasma concentrations demonstrated wide ranges, possibly due to either varying sampling times from the arrest-resuscitation event (the time from resuscitation to the first blood sample ranged from 2 h 10 min to 4 h and 45 min), differences in the severity of the ischemic insult, for example, time to restoration of circulation, prolonged post-resuscitation hypotension, or the effect of post-resuscitation therapy, for example, catecholamine infusions. Genetic polymorphism may have also played a role in the variability of the inflammatory response.

We observed an abrupt increase in TNF- α and IL-1 β shortly after reperfusion in our model of cardiac arrest and resuscitation, reminiscent of the response observed following injection of lipopolysaccharide or intact bacteria for the study of experimental sepsis (Michie and others 1988; Martich and others 1991; Casey and others 1993; Webel and others 1997; Gawad and others 2001). The rapid peak 30 min after reperfusion was followed by an expected sharp decline most likely due to cellular receptor binding, clearance by the kidney and liver, and possibly down-regulation by other cytokines or inflammatory mediators. Although TNF- α has

TABLE 1. CYTOKINE REGRESSION RESULTS

Time or gender	TNF- α			IL-1			IL-6		
	Estimate ^a	95% CI ^b	Adj. P value ^c	Estimate ^a	95% CI ^b	Adj. P value ^c	Estimate ^a	95% CI ^b	Adj. P value ^c
15	5.0	3.0–8.3	<0.0001	3.7	1.5–9.4	0.054	1.7	0.4–7.6	1.00
30	5.2	2.7–9.8	<0.0001	6.0	1.8–19.7	0.029	5.7	0.8–39.3	0.46
60	3.4	1.7–6.9	0.0065	8.8	2.4–32.2	0.011	5.8	0.7–48.5	0.55
90	3.5	1.7–6.9	0.0059	7.8	2.1–28.6	0.019	17.2	2.1–142.7	0.071
120	2.9	1.4–5.8	0.029	11.3	3.1–41.6	0.0031	17.4	2.1–144.0	0.069
180	2.1	1.0–4.4	0.32	11.9	3.0–47.1	0.0050	17.7	1.9–165.8	0.098
240	2.0	0.9–4.2	0.42	4.7	1.2–18.8	0.19	8.2	0.9–76.8	0.40
300	1.6	0.8–3.3	0.84	2.3	0.6–9.0	0.87	3.5	0.4–32.5	0.91
360	1.5	0.7–3.2	0.90	4.2	2.0–16.7	0.27	1.5	0.2–14.3	1.00
Male	4.0	2.9–5.5	<0.0001	0.9	0.5–1.6	0.64	1.2	0.4–3.2	0.72

^aAll estimates for time and gender effects are expressed as multiplicative factors, relative to a female swine at time 0 (baseline). Values are based on fixed effects, multivariable linear regression models of log-transformed cytokine measurements (see text).

^b95 % CIs for regression-based estimates of time and gender effects are not corrected for multiple comparisons.

^cReported *P* values are adjusted for multiple comparisons, with each comparison being made to baseline (time 0, female), using the Dunnnett–Hsu adjustment in SAS Proc Mixed. Thus, the unadjusted 95% CI may not cross 1.0, suggesting an unadjusted *P* value that is statistically significant, while the adjusted *P* value shows no such significance.

Abbreviations: CI, confidence interval; IL, interleukin; TNF, tumor necrosis factor.

a half-life of 20–40 min, plasma concentrations remained elevated for the first 3 h of reperfusion. Therefore, if initial sampling were done beyond this initial reperfusion period, the early increase in TNF- α after resuscitation would not likely be detected. IL-1 β concentrations increased in parallel to

that of TNF- α during early reperfusion but observed elevations persisted throughout the 6-h observation period. IL-1 β itself stimulates its own gene expression. Peak values were observed at 120 min. Variations in these cytokines were not seen in sham control animals.

TNF- α and IL-1 β both depress myocardial function and decrease systemic vascular resistance, resulting in arterial hypotension and may play a role in early post-resuscitation hemodynamic depression (Cain and others 1999; Dinarello 2000). We observed both profound hypotension and myocardial depression following reperfusion, which persisted throughout the observation period. The preliminary post hoc study evaluating the effect of infliximab on LV function suggests that TNF- α plays a role in early post-resuscitation myocardial dysfunction. The persistent depression of LV function, reflected in stroke work, during the recovery period is likely due to the myocardial infarction produced by balloon occlusion of the LAD. Total LAD occlusion exceeded 60 min in all surviving animals. Occlusion of this duration is likely to have resulted in irreversible injury to a large segment of the LV myocardial wall, resulting in a decline in indices of cardiac function that would be persistent, regardless of cytokine levels. For these reasons, the effects of infliximab on cardiac function were evaluated in a different, but conventional model of swine cardiac arrest involving electrical stimulation of the endocardium to produce ventricular fibrillation. Although the post-recovery TNF- α response is

FIG. 5. Temporal plasma tumor necrosis factor- α (TNF- α) concentration by gender and by 17-estradiol level. Significant differences between testosterone and 17-estradiol were not observed between sexes in these sexually immature swine. As noted earlier, the time dependence of TNF- α was highly significantly different when swine were separated by gender (*top panel*). There was no such difference when the swine were separated by estrogen level (low <20 pg/mL vs. high $\geq$ 20 pg/mL).

FIG. 6. Left ventricular (LV) stroke work control versus infliximab treatment. Infliximab infused over 30 min after return of circulation significantly improved stroke work when compared to normal saline. LV stroke work was significantly different at 30 min. * $P < 0.001$.

less pronounced in the electrical induction model (Niemann and others 2008), we observed that infliximab attenuated the early decline in cardiac function in this model.

Sexual dimorphism in the inflammatory response to physiologic stress has been previously reported in humans and animals. Included among these stressors are sepsis, trauma, burns, hemorrhage, and myocardial ischemia (Angele and others 1999; Angele and others 2000; Hessen and others 2002; Bouman and others 2004; Imahara and others 2005; Kher and others 2005; Martin and others 1997). Of note, several retrospective cohort studies of outcome following out-of-hospital cardiac arrest suggest that female gender is associated with increased survival (Kim and others 2001; Herlitz and others 2004; Arrich and others 2006). In our study, peak TNF- α concentrations were greater in males than females but both sexes demonstrated similar temporal

FIG. 7. Tumor necrosis factor- α (TNF- α) concentrations in control and infliximab groups. Plasma TNF- α concentrations were not different between the control and infliximab-treated study groups.

trends. However, sex steroid levels were similar for males and females, supporting prior observations in our laboratory (Niemann and others 2008). In the present study, we demonstrated that TNF- α levels were not different between high and low 17- α estradiol groups regardless of gender.

This study has several limitations. Although significant changes in plasma concentrations of TNF- α and IL-1 β were observed in this study, the ELISA method used for measurements does not discriminate between active and inactive or neutralized forms and endogenous antagonists were not measured. Importantly, the assays used do not detect precursor forms of either cytokine. Although bioassays would be of value of determining cytokine activities, they are limited by a relative lack of specificity (Cannon and others 1993). There appears to be an inverse relationship between TNF- α concentrations and cardiac function and infliximab lessened the degree of postarrest cardiac dysfunction. However, the post hoc study data can only be considered preliminary as only a single dose and infusion rate were used in a non-blinded manner. Nonetheless, improved cardiac function was observed even in a study with a small sample size. Although specific porcine anti-TNF- α antibodies were not used, porcine TNF shares a similar structure with that of human and murine TNF. Only 18 amino acids of the peptide sequence of porcine TNF- α are different from those in the sequence of man (Pauli 1995). The neutralization rate of human and porcine TNF- α by murine monoclonal anti-human TNF- α is 100% using the PK(15) assay, and human soluble TNF- α receptors bind porcine TNF- α (Pauli and others 1989; Pauli and others 1994). Infliximab is a chimeric human/mouse anti-TNF- α monoclonal antibody and there is no reason to suspect that the neutralization rate would not nearly complete. In the small number of animals that survived to the 6-h time, although adequate in number to allow analysis of the group as a whole, the small sample size limits comparisons between genders and may have contributed to the variability observed in cytokine concentrations. Amiodarone and epinephrine administered during resuscitation efforts may also have affected cytokines levels. Catecholamines have been shown to suppress cytokine production and the effects of amiodarone are unclear (van der Pool and others 1996; Matsumori and others 1997; van der Pool and Lowry 1997; Oral and others 1999). However, all animals received epinephrine, the administered dose was not different between males and females, and nearly equal numbers of males and females received amiodarone. Lastly, "downstream" effects of the measured cytokines, for instance, apoptosis, or leukocyte-mediated cell injury, were not evaluated (Moens and others 2005).

This study demonstrates that there is a prompt and dramatic increase in TNF- α and IL-1 β following resuscitation from prolonged cardiac arrest. This increase in cytokines is temporally related to a dramatic decline in cardiac function and persists for hours after resuscitation. The effect of neutralizing antibodies to TNF- α and IL-1 β on resuscitation outcome would further define the role of cytokines in the post-resuscitation syndrome.

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Author Disclosure Statement

None of the authors has a potential conflict of interest in connection with the submitted manuscript.

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