Resuscitation (2008) **77**, 401—409

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/resuscitation

EXPERIMENTAL PAPER

Myocardial cytokine IL-8 and nitric oxide synthase activity during and after resuscitation: Preliminary observations in regards to post-resuscitation myocardial dysfunction

Karl B. Kern*, Robert A. Berg, Ronald W. Hilwig, Douglas F. Larson, Mohamed A. Gaballa

Sarver Heart Center, University of Arizona College of Medicine, 1501 N. Campbell, Tucson, AZ 85724, USA

Received 31 August 2007; received in revised form 12 January 2008; accepted 22 January 2008

KEYWORDS
Cardiopulmonary resuscitation (CPR);
Nitric oxide;
Post-resuscitation period;
Stunning;
Myocardial

Summary
Aim: Increases in serum cytokines have been reported after successful resuscitation from prolonged ventricular fibrillation (VF). Pro-inflammatory cytokines can stimulate inducible nitric oxide synthase (iNOS) to produce excessive levels of nitric oxide (NO). High levels of both myocardial inflammatory cytokines and nitric oxide levels can depress myocardial contractile function. We hypothesized that myocardial pro-inflammatory cytokines and iNOS activity would increase following successful resuscitation from prolonged ventricular fibrillation cardiac arrest, and that such increases would parallel the development of post-resuscitation myocardial dysfunction.
Methods: Ventricular fibrillation cardiac arrest was induced in seven domestic swine (25 ± 5 kg). After 10 min of untreated VF, the animals were defibrillated and resuscitated. Left ventricular (LV) systolic and diastolic function measurements, serum samples (arterial and coronary sinus) for IL-8 cytokine quantification, and LV myocardial biopsies were collected before, during, and after resuscitation. Quantification of myocardial endothelial (eNOS) and inducible (iNOS) nitric oxide synthase protein levels were determined using immunoblot analyses and protein localization was examined using immunohistochemistry.
Results: Post-resuscitation LV systolic and diastolic functions were depressed while increases in both coronary sinus IL-8 levels and myocardial iNOS activity were found. Compared to pre-arrest baseline, levels of iNOS protein increased during VF ($p \leq 0.05$) and continued to increase throughout the post-resuscitation study period of 6 h ($p \leq 0.05$).

* Corresponding author. Tel.: +1 520 626 2477; fax: +1 520 626 4333.
E-mail address: kernk@email.arizona.edu (K.B. Kern).

Conclusions: Myocardial inflammatory cytokines and iNOS activity increase during and after prolonged cardiac arrest and successful resuscitation. These increases correspond to the well described decrease in LV function post-resuscitation.

Introduction

Initial resuscitation rates from out-of-hospital cardiac arrest can be 40% or better, particularly if rapid defibrillation is available. Unfortunately, the long-term survival of such patients remains poor, even after initial successful resuscitation, in part because of post-resuscitation myocardial dysfunction.[1–3] In spite of its importance, our understanding of the etiology of such systolic and diastolic myocardial dysfunction post-resuscitation remains limited.

Increases in pro-inflammatory cytokines (including IL-1β, IL-8 and TNFα) have been found prompting Adrie et al. to suggest that successful cardiac resuscitation creates a ''Sepsis-Like'' syndrome.[4] Myocardial dysfunction during sepsis is well described,[5–7] and has been linked to the presence of a circulating 'myocardial depressant substance'. The most likely candidates for such 'myocardial depressant substances' are inflammatory cytokines and/or nitric oxide (NO).[8–13] Data during sepsis suggest that inflammatory cytokines and NO may work synergistically to depress left ventricular function.[14,15]

Nitric oxide is produced from L-arginine by a group of NO synthases (NOS). In the cardiac tissue, NO is produced by three isoforms of nitric oxide synthase, endothelial nitric oxide (eNOS), inducible nitric oxide (iNOS), and neuronal nitric oxide (nNOS). Stimulation of eNOS, a calcium-dependent enzyme, typically produces small amounts of NO, while stimulation of iNOS, a calcium-independent enzyme, can produce 10-fold higher levels of NO compared with eNOS. Pro-inflammatory cytokines have been shown to stimulate myocardial iNOS protein production.[16] Increased iNOS activity and protein expression has been associated with chronic heart failure.[17–19] Excessive increases in myocardial NO levels from stimulated iNOS may suppress left ventricular function.[20]

We hypothesized that following successful resuscitation, myocardial production and activity of the cytokine IL-8 and iNOS would increase and that such increases would parallel the development of post-resuscitation myocardial dysfunction.

Materials and methods

Animal preparation

This study was conducted with the approval of the University of Arizona Institutional Animal Care and Use Committee in accordance with the guidelines set forth in the *Position of the American Heart Association on Research Animal Use*. Seven healthy, young domestic swine (25—35 kg) of either sex (five females, two males) were anesthetized with 5% isoflurane inhalation anesthetic in oxygen delivered by nose cone mask. The animals were intubated and anesthesia was maintained with 1.5—3% isoflurane in room air, until the induction of ventricular fibrillation, using a rate- and volume-regulated ventilator/anesthesia machine (Narkomed 2A ventilator, North American Drager, Inc.). Initially, breaths were given at 12/min with a tidal volume (TV) of 15 ml/kg. Rate and/or TV was subsequently adjusted to maintain expired end-tidal carbon dioxide ($ETCO_2$) at 40 ± 3 mmHg (mean $\pm$ S.D.), as monitored by an infrared capnometer (Hewlett Packard 47210A, Palo Alto, CA) and a pneumotachometer (MP45-871, Validyne Engineering Corp, Northridge, CA) placed in the airway. Electrocardiographic leads were attached to all limbs to monitor heart rate (HR) and the electrocardiogram (ECG). Vascular introducer sheaths (5 or 7F, Cordis Corp, Miami, FL) were placed in the right external and both internal jugular veins, the right carotid artery, and right femoral artery by standard cut-down procedure. Right atrial and aortic pressures were measured with solid-state micro-tipped transducers (P-500, Millar Instruments, Houston, TX). The left ventricular pressure was measured with a solid-state micro-tipped transducer (P-500, Millar Instruments, Houston, TX) for subsequent calculation of the first derivative of LV pressure overtime (dP/dt and $-dP/dt$), and the time course of LV isovolumic relaxation (tau). Occasionally, this solid-state micromanometer was replaced with a 7F fluid-filled pig-tail catheter (Cordis Corp, Miami, FL) to allow injection of contrast into the left ventricle (LV) for the calculation of left ventricular ejection fraction (EF). Blood was also collected periodically via this pigtail catheter for laboratory analyses of cytokine content in the arterial system. A standard myocardial bioptome (Cordis Corp, Miami, FL) was placed retrograde across the aortic valve into the LV cavity, via the carotid artery sheath, to obtain endomyocardial tissue samples for later immunohistochemical analyses. Endomyocardial samples (1 mm^3) were obtained and immediately frozen in liquid nitrogen, as previously reported from our laboratory.[21] A balloon-tipped pulmonary artery catheter was placed via an internal jugular vein into the right pulmonary artery for measuring cardiac output. A fluid-filled catheter was placed, via the other internal jugular vein, into the coronary sinus to periodically collect blood for analyses of cytokine content. Catheter/transducer placements were confirmed by fluoroscopy.

Measurements

Hemodynamic measurements

Hemodynamic data, including pressures from the aorta (AoP), right atrium (RAP), the left ventricular change in pressure overtime (dP/dt), negative dP/dt, and tau, were displayed and recorded ((D1-220-PGH, Dataq Instruments Inc, Akron, OH). In addition, $ETCO_2$, ECG and tidal volume were continuously displayed and recorded using the same commercially available data collection software.