

NHE-1 Inhibitors and Erythropoietin for Maintaining Myocardial Function during Cardiopulmonary Resuscitation

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ABSTRACT

Efforts to successfully restore life in cardiac arrest victims are formidably challenging. They require not only that cardiac activity be initially reestablished but that injury to vital organs be prevented or minimized. In this article, we discuss the effects that cardiac arrest and resuscitation have on the myocardium, describing first the functional myocardial abnormalities that occur during cardiac resuscitation, which may limit the ability to reestablish cardiac activity. We then discuss strategies for minimizing myocardial injury and examine novel therapies aimed at minimizing ischemia and reperfusion injury. Finally, we discuss sodium-hydrogen exchanger isoform-1 (NHE-1) inhibitors and erythropoietin for maintaining myocardial function during cardiopulmonary resuscitation.

Keywords: myocardial ischemic injury, reperfusion myocardial injury, cardiopulmonary resuscitation, NHE-1 inhibitors, erythropoietin

Myocardial Ischemic Injury during Cardiac Resuscitation

The working heart is a highly metabolic organ that under normal resting conditions extracts nearly 70% of the oxygen supplied by the coronary circulation (1,2) representing close to 10% of the total body oxygen consumption. However, the heart has minimal capability for extracting additional oxygen, such that increases in metabolic demands can only be met by autoregulatory increases in coronary blood flow through vasodilatation of the coronary circuit. (3) Consequently, a severe energy imbalance

develops when cardiac arrest occurs and coronary blood flow ceases. The severe energy imbalance continues during the ensuing resuscitation effort when current closed-chest resuscitation techniques are used because of the very limited capability for generating systemic and coronary blood flow. (4) The magnitude of the energy imbalance is contingent on the metabolic requirements and is particularly severe in the presence of ventricular fibrillation (VF) when the oxygen requirements are comparable to or exceed those of the normally beating heart. (5,6) A lesser energy deficit is expected during cardiac arrest with a quiescent or minimally active heart (i.e., asystole or pulseless electrical activity precipitated by asphyxia or exsanguinations). Moreover, with reperfusion during resuscitation, multiple pathogenic mechanisms – collectively known as “reperfusion

injury” – are activated and further contribute to myocardial injury. Main contributors to reperfusion injury are mitochondrial Ca^{2+} overload (7,8) and generation of reactive oxygen species (ROS). (9)

The limited oxygen supply and concomitant reperfusion injury compromise the mitochondrial capability for regenerating ATP (adenosine triphosphate) through oxidative phosphorylation. Limited amounts of ATP; however, are generated at the substrate level from anaerobic glycolysis and breakdown of creatine phosphate. Taking these processes together, the myocardium develops a marked increase in lactic acid, rapid depletion of creatine phosphate, and relatively slow depletion of ATP during cardiac arrest and resuscitation. (10) Accordingly, the resuscitation effort typically proceeds – and occasionally succeeds – in the pre-

sence of ischemia and in the midst of reperfusion injury.

Various functional myocardial abnormalities develop, consequent to ischemia and reperfusion, during cardiac arrest and resuscitation that exert effects detrimental to cardiac resuscitation. These abnormalities can be grouped into those that manifest during the resuscitation effort and those that manifest after the return of spontaneous circulation. The former include reductions in left ventricular myocardial distensibility and increased resistance to electrical defibrillation; the latter include reperfusion arrhythmias and post-resuscitation myocardial dysfunction. This discussion is focused on reductions in left ventricular myocardial distensibility and the extent to which its prevention can improve resuscitation and survival.

Reductions in Left Ventricular Myocardial Distensibility during Cardiac Resuscitation

Preclinical evidence

Studies in various animal models of VF and resuscitation have shown progressive thickening of the left ventricular wall accompanied by parallel reductions in the left ventricular cavity without changes in intracavitary pressures during the resuscitation effort. (11,12) A functionally similar phenomenon – known as ischemic contracture – was reported in the early seventies during open heart surgery when operations were conducted under normothermic conditions and in fibrillating hearts (13,14) and more recently after prolonged intervals of untreated VF. (15) However, ischemic contracture is associated with profound reductions in myocardial ATP and often leads to a “stony heart” heralding irreversible ischemic injury. (16)

Reductions in left ventricular myocardial distensibility, observed during cardiac resuscitation, is a different phenomenon: 1) it occurs much earlier than the “stony heart”, 2) the onset and subsequent progression coincide with the interval of reperfusion during resus-

citation, (11,17) 3) it is associated with less ATP depletion, (10) 4) it has been attributed to myocardial energy deficit compounded by cytosolic and mitochondrial Ca^{2+} overload precluding complete relaxation of individual cardiomyocytes, 5) it evolves into diastolic dysfunction upon return of spontaneous circulation, (18) and 6) it is largely reversible. (19)

There are important hemodynamic consequences. As blood returns to the heart during the relaxation phase of chest compression, distensible ventricles are important to properly accommodate the returning blood and establish an adequate preload for the subsequent compression. The larger the distensibility, the larger the preload, and the larger the amount of blood that can be ejected by chest compression. This mechanism is akin to the Frank-Starling Law of the beating heart and presumes that blood is ejected from the left ventricle into the aorta during chest compression. Several experimental and clinical studies – using echocardiography – have documented that the heart is indeed compressed between the anterior chest wall and the spine and that during compression the mitral valve closes and the aortic valve opens. (20-22)

Progressive decreases in left ventricular myocardial distensibility during chest compression contribute to progressive decline in the hemodynamic efficacy of closed-chest resuscitation. Studies in a porcine model of VF have shown that the severity of this phenomenon is proportional to the duration of untreated VF. (11)

Work in our laboratory demonstrates that reductions in left ventricular myocardial distensibility can be prevented by pharmacologic interventions targeting reperfusion injury resulting in hemodynamically more stable closed-chest resuscitation. (17,23) In our work we showed that progressive left ventricular wall thickening with reductions in cavity size were mitigated by administration of the sodium-hydrogen exchanger isoform-1 (NHE-1) inhibitor cariporide. (17) This effect prevented the hemodynamic deterioration that

characteristically occurs during chest compression maintaining a stable coronary perfusion pressure above the resuscitability threshold of 10 mmHg in pigs yielding higher resuscitation rates. (17)

Clinical evidence

Takino and Okada (24) reported in 1996 on 59 adult patients who suffered non-traumatic out-of-hospital cardiac arrest and underwent open-chest direct manual cardiac compression in the emergency department after failure of closed-chest resuscitation. A “firm” myocardium was noticed during manual cardiac compression in 36 cases affecting predominantly the left ventricle. In the remaining 23 cases the hearts were “soft.” They also noted that some hearts became “firm” during compression.

The presence of a “firm” myocardium was associated with reduced hemodynamic efficacy of cardiac compression as evidenced by a lower end-tidal CO_2 tension (PETCO₂) – which is a well documented surrogate measurement of systemic and regional blood flow during cardiac resuscitation. (4,25-27) Hearts with “very firm” myocardium never regained spontaneous contractions. Hearts with “less firm” myocardium showed some, albeit insufficient, spontaneous contractions. Hearts with “soft” myocardium regained contractions and were able to generate a peripheral pulse in most instances.

Other Myocardial Abnormalities during Resuscitation

Notwithstanding the focus of the present discussion on left ventricular myocardial distensibility, the abnormalities succinctly described below share a common pathophysiological thread. Thus, interventions exerting an effect on left ventricular myocardial distensibility could also affect:

- 1) resistance to defibrillation (29)
- 2) reperfusion arrhythmia (30)
- 3) post-resuscitation myocardial dysfunction as previously reported to be the case associated with administration of NHE-1 inhibitors. (31-35)

Work in our research laboratory and

subsequent clinical work in collaboration with Dr. Štefek Grmec, MD, PhD in Maribor, Slovenia demonstrates that these myocardial abnormalities in left ventricular dysfunction can be ameliorated by pharmacological intervention. We propose that this approach may represent a novel concept in resuscitation leading to new therapeutic interventions.

Therapeutic Interventions

Two lines of research, pointing to inhibitors of the NHE-1 and to erythropoietin as supporting the feasibility of the proposed approach, are discussed in this section. One line relates to work using NHE-1 inhibitors in various animal models of cardiac arrest over a period of approximately 10 years. (10,17,23,28,36-55) Research over the last decade in our laboratory using various translational rat and pig models of cardiac arrest has shown consistent myocardial benefit associated with inhibition of NHE-1 activity during resuscitation from VF. (10,17,23,28,37-41,56-60) Mechanistically, these benefits are associated with less cytosolic Na⁺ overload, less mitochondrial Ca²⁺ overload, and preservation of oxidative phosphorylation. The other relates to more recent work using erythropoietin in a rat model of cardiac arrest (42) and in a small clinical study in patients suffering out-of-hospital cardiac arrest. (43) Both lines of research support the rationale and feasibility of using either an NHE-1 inhibitor or erythropoietin for preservation of left ventricular myocardial distensibility during cardiac resuscitation.

Effects of NHE-1 inhibition on resuscitation: Research over the last decade in our laboratory using various translational rat and pig models of cardiac arrest has shown consistent myocardial benefit associated with inhibition of NHE-1 activity during resuscitation from VF. (10,17,23,28,37-41,56-60) Mechanistically, these benefits are associated with less cytosolic Na⁺ overload, less mitochondrial Ca²⁺ overload, and preservation of oxidative phosphorylation. Some of these studies, highlighting

key aspects of NHE-1 inhibition during resuscitation, are succinctly discussed below.

The initial findings suggesting that NHE-1 inhibition could attenuate reductions in left ventricular myocardial distensibility during resuscitation and also prevent post-resuscitation diastolic dysfunction were made in an isolated (Langendorff) rat model of VF and simulated resuscitation. (37,38)

Subsequent studies were conducted in an intact rat model of VF and closed-chest resuscitation and these studies also suggested that higher coronary perfusion pressures could be generated when administering a vasopressor agent given the larger blood flow generated in the presence of an NHE-1 inhibitor for a given compression depth. This was the case when cariporide was combined with epinephrine in our pig model (28) and when combined with epinephrine and with vasopressin in our rat model. (40)

Like in previous studies which used the NHE-1 inhibitor cariporide, (17) zoniopride also prevented reductions in left ventricular myocardial distensibility during the interval of VF and extracorporeal circulation, which in control pigs was characterized by progressive reductions in cavity size and progressive thickening of the left ventricular wall. These energy effects are consistent with NHE-1 inhibition protecting mitochondrial bioenergetic function – probably as a result of limiting mitochondrial Ca²⁺ overload – and supportive of the concept that left ventricular myocardial distensibility during resuscitation is likely to be preserved by activating mitochondrial mechanisms capable of maintaining bioenergetic function.

Erythropoietin

Cell mechanism: Erythropoietin is a 30.4-kDa glycoprotein best known for its action on erythroid progenitor cells and regulation of circulating red cell mass. However, several studies have recently shown that erythropoietin also activates potent cell survival mechanisms during ischemia and reperfusion through genomic and non-genomic

signaling pathways in a broad array of organs and tissues including the heart, (61-66) brain, (67,68) spinal cord, (69) retina, (70) kidney, (71) liver, (72) and skin. (73)

Activation of these protective mechanisms involves binding of erythropoietin to a specific cell membrane receptor, member of the type-I superfamily of single-transmembrane cytokine receptors, prompting cross-phosphorylation and activation of Janus tyrosine kinases (JAK) 1 and 2. Activation of JAK causes phosphorylation of tyrosine residues creating docking sites for the recruitment and activation of multiple signaling proteins that have Src-homology-2 (SH2) domains resulting in well-established anti-apoptotic, (62) anti-inflammatory, (74,75) and proliferative effects (i.e., neovascularization) (76,77) with time courses that vary contingent on the specific signaling mechanism and the duration of the erythropoietin binding to the EpoR (erythropoietin receptors).

Although important in other settings, these effects are not likely to play a role for initial cardiac resuscitation. We hypothesize that erythropoietin is important for resuscitation by signaling through pathways that result in preservation of mitochondrial bioenergetic function leading to functional effects similar to those elicited by NHE-1 inhibition (albeit through quite distinct cell mechanisms). These mechanisms – we hypothesize – involve activation of protein kinase C epsilon (PKCε) and protein kinase B (Akt) as succinctly described below.

PKCε activation is a well established mechanism of myocardial protection believed to be responsible for preconditioning and acute protection. (78) PKCε is primarily located in the cytosol. Its phosphorylation by erythropoietin prompts translocation to the mitochondria where it could: 1) open putative mitochondrial ATP-sensitive K⁺ channels (KATP channels), (79,80) 2) activate cytochrome c oxidase, (81) 3) activate aldehyde dehydrogenase, (82) and 4) inhibit the mitochondrial permeability transition pore. (83) Opening of KATP channels increases mito-

chondrial K⁺ conductivity. This is an energetically advantageous (84,85) effect likely to limit mitochondrial Ca²⁺ overload (86) exerting effects similar to those of NHE-1 inhibition. Activation of cytochrome c oxidase (complex IV of the respiratory electron transport chain) could improve the efficiency of electron flow from cytochrome c to molecular oxygen enhancing the capability for ATP synthesis. Activation of aldehyde dehydrogenase (ADH2) could reduce the formation of toxic HNE-Michael adducts (4-hydroxy-trans-2-nonenal Michael adducts) and serve to preserve mitochondrial respiration.

Akt activation is a powerful survival signal that has been shown to mediate myocardial protection during late preconditioning and after reperfusion. (87) Erythropoietin mediates the phosphorylation of Akt through phosphorylation of phosphoinositide dependent kinase-1 (PDK1) upon activation of phosphatidylinositol kinase (PI3K). Activated Akt can translocate to mitochondria where it has been shown to exert beneficial effects including: 1) opening of mitochondrial K_{ATP} channels (87) with the anticipated effects as described above, 2) activation of respiratory chain complexes and FoF1 ATPase, (88) and 3) inhibition of the mitochondrial permeability transition pore. (89)

Effects on resuscitation

Studies in rats: The effects of erythropoietin were studied in our rat model of VF and closed-chest resuscitation using human recombinant erythropoietin (epoetin alpha, Amgen, Thousand Oaks, CA). (42) Rats were subjected to 10 minutes of untreated VF followed by 8 minutes of closed-chest resuscitation before attempting defibrillation. The depth of compression was adjusted to maintain an aortic diastolic pressure between 26 and 28 mmHg. This level of diastolic aortic pressure secured a coronary perfusion pressure above the resuscitability threshold of 20 mmHg in rats. The relationship between the coronary perfusion pressure and compression depth (CPP/Depth) was used

to assess changes in left ventricular myocardial distensibility. Successfully resuscitated rats were observed for 120 minutes before euthanasia.

Three groups of 10 rats each were randomized to receive a right atrial bolus of epoetin alpha (5,000 IU/kg) at baseline 15 minutes before induction of VF (EPOBL -15-min), at 10 minutes of VF before starting chest compression (EPOVF 10-min), or to receive 0.9% NaCl solution (control) instead with the investigators blind to the treatment assignment.

Erythropoietin given coincident with the beginning of chest compression after 10 minutes of untreated VF – but not before inducing VF – promoted hemodynamically more effective chest compression such that the CPP/Depth ratio averaged during the interval of chest compression was 2.0 ± 0.3 mmHg/mm in EPOVF 10-min, 1.6 ± 0.2 mmHg/mm in EPOBL -15-min, and 1.6 ± 0.3 mmHg/mm in the control group ($p < 0.05$ EPOVF 10-min vs EPOBL -15-min and vs control). This difference represented a 25% improvement in the hemodynamic efficacy of chest compression with erythropoietin given at the beginning of chest compression.

The possibility that this effect resulted from a vasopressor action of erythropoietin seemed unlikely; baseline hemodynamic measurements in the group of rats that received erythropoietin 15 minutes before induction of VF (EPOBL -15-min) demonstrated a statistically borderline decrease (not increase) in systemic vascular resistance from 1.092 ± 0.147 to 1.010 ± 0.133 mm Hg/mL/min/kg ($p = 0.077$ by paired t-test).

Defibrillation restored spontaneous circulation in 8 EPOBL -15-min, 8 EPOVF 10-min, and 9 controls. Post-resuscitation, EPOVF 10-min rats had significantly higher mean aortic pressure associated with numerically higher cardiac index and higher peripheral vascular resistance. The diminished effectiveness of erythropoietin when given before VF is intriguing and worthy of additional investigation.

Studies in humans: A clinical study was

performed in collaboration with Dr. Štefek Grmec, MD, PhD and colleagues at the Maribor Emergency Medical Services (EMS) system in the city of Maribor and adjacent rural areas encompassing a population of approximately 200,000 inhabitants. (43)

Resuscitation was attempted using regionally developed protocols that incorporate ILCOR 2005 recommendations by a two-tier system composed of basic life support (BLS) and advanced life support (ALS) teams with the latter led by a physician. Upon arrival of the ALS team the trachea was intubated – verifying proper placement by capnography – and positive pressure ventilation started with a tidal volume of approximately 6 ml/kg at 10 breaths per minute unsynchronized to compressions. The ALS team also established peripheral vascular access (within approximately 30 seconds). Patients assigned to erythropoietin received 90,000 IU of beta-epoetin (NeoRecorcom, Hoffman La Roche) as a bolus within 1 or 2 minutes after starting chest compression followed by a 10-ml bolus of 0.9% NaCl. Beta-epoetin was kept refrigerated (2-8 °C) in the ambulance until immediately before use. In every instance erythropoietin was given before any other drug.

Patients who had return of spontaneous circulation were started on 0.9% NaCl solution cooled at 4°C (30 ml/kg infused at 100 ml/min) and given 0.08-0.10 mg/kg of vecuronium bromide (Norcuron®, Organon) to initiate hypothermia while on route to the hospital. For hemodynamic stability, patients received dopamine (5-10 mcg/kg/min) for systolic blood pressure <90 mmHg, dobutamine (2.5-20.0 mcg/kg/min) for suspected cardiogenic shock, or if systolic blood pressure remained < 70 mmHg despite the preceding measures norepinephrine (8-12 mcg/kg/min).

Upon hospital arrival patients were directly admitted to the Intensive Care Unit (ICU). Patients were cooled to a core temperature between 32 and 34°C by external means until they regained consciousness or had completed 24

hours. Patients with ST-segment elevation myocardial infarction had percutaneous coronary interventions. Inotropic and vasopressor agents were infused guided by hemodynamic monitoring using a pulmonary artery catheter and transthoracic echocardiography.

Study groups: The study was originally designed as prospective and randomized. However, disruption in the supply of erythropoietin, prompted investigators to administer erythropoietin or 0.9% NaCl control based on availability, allocating 24 patients to erythropoietin and 30 to 0.9% NaCl between April 2007 and May 2008. The control group for the analysis was designated as concurrent controls. Post-hoc, a second control group was included selecting 48 patients out of 126 patients who had out-of-hospital cardiac arrest treated with the same resuscitation protocol the year before. These 48 patients were selected using propensity scores assigning two controls for each erythropoietin-treated patient. Propensity scores were calculated using multiple logistic regressions; entering age, male sex, witnessed arrest, time from call to start CPR (cardiopulmonary resuscitation), pulseless electrical activity, asystole, and bystander CPR as pre-treatment predictors of outcome. The control

group was designated as matched controls. The same variables used to calculate propensity scores were used to adjust odds ratios for the comparison between erythropoietin and the concurrent controls and between erythropoietin and the matched controls.

Results: By univariate analysis, administration of erythropoietin – when compared with concurrent controls – was associated with higher rates of ICU admission, ROSC (resuscitation of spontaneous circulation), 24-hour survival, and survival to hospital discharge and – when compared with matched controls – it was associated with higher rates of ICU admission, ROSC, and 24-hour survival. After adjustment for pretreatment covariates (listed above), comparison with concurrent controls reduced the odds ratio but retained statistical significance for ICU admission and ROSC whereas comparison with matched controls increased the odds ratio demonstrating statistical significance for all four outcomes).

To assess whether the beneficial effects on resuscitation outcomes could have been linked to the beneficial effects on left ventricular myocardial distensibility – as suggested by our preceding study in rats (42) – we examine the effects on PETCO₂. As discussed

earlier, PETCO₂ is a good surrogate measurement of forward blood flow during chest compression. (4,25-27) In the study, rescuers were trained and retrained to provide consistent depth and rate of compression and the values of PETCO₂ in both control groups were already indicative of high quality chest compression. If – as hypothesized – erythropoietin preserved myocardial distensibility, for a given compression depth, one would expect higher forward blood flow in the presence of erythropoietin and therefore higher P_{ET}CO₂. This was indeed the case. Victims who received erythropoietin had significantly higher P_{ET}CO₂ during chest compression.

Because a single dose was administered, substantial effects on erythropoiesis were not anticipated. In fact, the hemoglobin and hematocrit in the present study were not statistically different than controls at 48 hours and at 72 hours.

Accordingly, these clinical observations – though based on a small sample size – are consistent with the hypothesis that erythropoietin – by preserving myocardial distensibility – leads to hemodynamically more effective chest compression resulting in higher resuscitation and survival rates.

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