Ischemic Postconditioning during Cardiopulmonary Resuscitation Improves Acute Outcomes in a Porcine Model of Prolonged Cardiac Arrest

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Dedication

I dedicate this work to my mentors in life. To my parents, Joyce and John, for providing the means and encouragement to explore my curiosities. To my brothers, Brian and Michael, for their friendship, perspective, and intellectual stimulation in all stages of my development. And to Amy, for sharing with a physiologist the wonder of life outside of the laboratory.

Abstract

Cardiac arrest (CA) is the cessation of blood flow due to electrical or mechanical failure of the heart resulting in whole-body ischemia. Out-of-hospital CA (OHCA) is a medical emergency occurring in approximately 350,000 people in the United States each year with a survival rate of 5-15%. Mechanical improvements to cardiopulmonary resuscitation (CPR) as well as organizing a systems-based approach to resuscitation have resulted in only modest improvements in survival. Novel resuscitation strategies are necessary to improve survival following OHCA.

Two phases of injury are associated with prolonged ischemia: an initial injury during untreated ischemia, followed by paradoxical damage that occurs upon reperfusion. During CA, ischemic injury occurs prior to resuscitation efforts, whereas reperfusion injury begins when CPR is initiated. The extent and development of reperfusion injury during resuscitation following OHCA is poorly characterized.

Postconditioning, a suite of strategies applied after a lethal ischemia to mitigate reperfusion injury, can attenuate infarct size and organ dysfunction in the heart and brain when applied early during reperfusion. Following a CA, reperfusion is initiated with CPR. Thus, postconditioning strategies are predicted to have the greatest efficacy when applied during CPR for the treatment of CA. Ischemic postconditioning (IPC), a technique of non-lethal pauses in blood flow during early reperfusion, has demonstrated protection of vital organs after focal ischemia. The ability to protect multiple organs makes IPC an appealing therapeutic for the whole-body ischemia of CA. Further, IPC is ideal for CA because controlled pauses in reperfusion can be accomplished simply by interrupting continuous chest compressions.

The central hypothesis of this work is that IPC implemented at the initiation of CPR can improve cardiac and neurologic recovery after prolonged CA. This work details experiments investigating the feasibility and efficacy of implementing a simple IPC strategy at the onset of CPR to mitigate organ and mitochondrial dysfunction following prolonged ventricular fibrillation (VF) CA. In a porcine model, IPC drastically improved cardiac function and neurologically favorable survival after 15 minutes of VF CA. IPC also

improved hemodynamics during CPR and increased cardiac mitochondrial function in the acute phase of resuscitation. A combination of IPC with other postconditioning strategies promoted cardiac and neurologic recovery after 17 minutes of untreated VF CA, a duration not previously compatible with positive outcomes in a porcine model. The mechanisms that mediate cardiac and neurologic protection remain undetermined, though evidence suggests increased coronary perfusion pressure during resuscitation is necessary and sufficient to restore acute cardiac mitochondrial function after prolonged VF CA.

These experiments highlight the impact of early CPR interventions on long-term outcomes, and emphasize the importance of continued innovation in resuscitation therapies. Every incremental improvement to resuscitation care has the potential to save thousands of lives.

Contributions

In addition to authors explicitly named in each chapter, the following people contributed significantly to the work described herein:

• Michael Lick contributed to the work in Chapter 3 – Part 2 as a research technician in surgical preparation and data management.
• Rachel Stark contributed to the work in Chapters 4 and 5 in animal care and as a surgical technician. Her efforts were paramount to the success of those experiments.
• Qunli Cheng, MD and Mohammed Aldakkak, MD from the Medical College of Wisconsin were instrumental in the development and instruction in the use of the mitochondrial functional assays used during experiments described in Chapters 4 and 5.

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CHAPTER 1 – Part 1

Overview

Cardiac Arrest and Cardiopulmonary Resuscitation

Cardiac arrest is the cessation of blood flow due to electrical or mechanical failure of the heart resulting in whole-body ischemia. Out-of-hospital cardiac arrest (OHCA) occurs approximately 350,000 times annually in the United States (1). Outcomes following OHCA are poor and represent a heavy burden in terms of mortality and morbidity (2).

Since efficacy of external cardiac massage was demonstrated by Kouwenhoven et al., cardiopulmonary resuscitation (CPR) has been the predominant initial therapy to reverse cardiac arrest (3). In the nearly six decades since, permutations of mechanical manipulations have been attempted to improve blood pressure during CPR. Evidence of a thoracic pump (4) paved the way for intrathoracic pressure regulation and active compression-decompression (5,6). These insights have now been engineered into devices that can facilitate sustained, high-quality CPR. In contrast to vasopressor dogma, sodium nitroprusside (SNP) in tandem with mechanical interventions to centralize blood flow was shown to increase vital organ perfusion as well as neurologically favorable survival (7). Extracorporeal membrane oxygenation (ECMO) represents the zenith of artificial cardiac output and shows potential for increased survival following OHCA (8,9).

However, improved techniques to generate blood flow have thus far failed to reliably and substantially improve outcomes from OHCA. Evidence from ischemia in isolated organs indicates that reperfusion injury, the paradoxical damage that occurs upon introduction of blood flow after a long bout of ischemia, contributes significantly to cellular and organ damage following prolonged ischemia (10). Postconditioning is a term for a suite of therapeutic strategies applied after a lethal ischemia to limit injury by mitigating reperfusion injury. Ischemic postconditioning (IPC), a technique of administering non-lethal controlled pauses in blood flow during early reperfusion, has demonstrated protection via attenuation of infarct size, apoptosis, and organ dysfunction in the heart,

brain, and other vital organs (10–16).

Postconditioning shows the greatest efficacy when applied early during reperfusion. Following a cardiac arrest, reperfusion is initiated with CPR. Thus, postconditioning strategies are predicted to have the greatest efficacy when applied during CPR for the treatment of cardiac arrest. The ability to protect multiple organs makes IPC an appealing therapeutic for the whole-body ischemia of cardiac arrest. IPC is more relevant than preconditioning for the treatment of cardiac arrest due to the unpredictability of an arrest. Further, IPC is an ideal therapeutic because controlled bouts of ischemia/reperfusion can be accomplished easily during early reperfusion with CPR by temporarily discontinuing chest compressions. For these reasons, IPC was implemented as a therapeutic strategy to investigate the potential of postconditioning during CPR to improve survival and vital organ function after prolonged cardiac arrest.

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Objectives

The following aims were developed to address the safety and efficacy of IPC when applied during CPR (IPC-CPR) in a porcine model of prolonged ventricular fibrillation (VF) cardiac arrest.

1) Determine the feasibility and efficacy of IPC-CPR to promote cardiac and neural recovery in a porcine model of prolonged VF cardiac arrest.
2) Determine the synergy of IPC with other postconditioning strategies during CPR.

In Chapter 2, we tested the feasibility and efficacy of implementing IPC at the initiation of CPR via controlled pauses in chest compressions and ventilations during the first few minutes of CPR. We hypothesized that implementation of an IPC strategy during the initiation of CPR would improve cardiac and cerebral recovery after 15 minutes of VF cardiac arrest.

In Chapter 3, we investigated the effects of IPC ± vasodilators at the initiation of CPR on cardiac and cerebral functional after 15 minutes of VF cardiac arrest. We hypothesized that vasodilator therapy would act synergistically with IPC to protect the

heart and brain. We also investigated the effects of combining IPC with two other strategies, anesthetic postconditioning with sevoflurane and pharmacologic postconditioning with P188, at the initiation of CPR on cardiac and cerebral function after 17 min of VF cardiac arrest. We hypothesized that a combination of postconditioning strategies would provide synergistic benefit to prevent sever injury, thus promoting cardiac and neurologic recovery after a duration of untreated VF cardiac arrest never before associated with positive outcomes.

Results from Aims 1 and 2 confirmed cardiac and neural protection via IPC-CPR following prolonged cardiac arrest. Based on these findings, we next focused on determining the role of mitochondria in mediating this protective benefit. Mitochondria are a nexus for integrating signals of both injury and protection during reperfusion and postconditioning, respectively (17,18). The review included in this chapter summarizes the rationale and recent advances for the use of postconditioning strategies to treat cardiac arrest.

The following aims were developed to determine the effect of IPC-CPR on acute mitochondrial function, and to determine the role of enhanced coronary perfusion pressure as a mediator of mitochondrial protection conferred by IPC-CPR.

3) Characterize the effect of cardiac arrest, standard CPR, and IPC-CPR on mitochondrial function in the vital organs of the heart and brain in a porcine model of prolonged VF cardiac arrest.
4) Investigate the effect of increased coronary perfusion pressures on acute cardiac mitochondrial function during resuscitation with and without IPC.

In Chapter 4, we characterized the severity of mitochondrial dysfunction in the heart and brain during cardiac arrest and standard CPR, and determined the potential for IPC to rescue mitochondrial function after 15 minutes of VF cardiac arrest. We hypothesized that cardiac and brain mitochondrial dysfunction develops during prolonged cardiac arrest and

can be rescued with IPC-CPR. We also characterized mitochondrial function six hours after return of spontaneous circulation. We hypothesized that differences in acute mitochondrial function between treatments would persist during recovery from prolonged cardiac arrest.

In Chapter 5, we investigated the effect of coronary perfusion pressure during resuscitation on acute mitochondrial function. We hypothesized that IPC-CPR confers benefit to cardiac mitochondrial function via increased coronary perfusion pressure.

Chapter 1 – Part 2

Post-conditioning to improve cardiopulmonary resuscitation

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Purpose of review

Despite decades of advances in prehospital and in-hospital medical care, patients with out-of-hospital cardiac arrest continue to have poor neurologic and cardiac function following otherwise successful resuscitation. This review examines the mechanisms and therapeutic strategies currently under development to activate the post-conditioning pathways and thereby improve survival and function.

Recent findings

Post-conditioning utilizes the reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) pathways as common avenues to promote cell survival and function. Ischemic postconditioning and multiple medications activate these pathways resulting in improved cardiac and neurological function in animal models of cardiac arrest.

Summary

Detailed knowledge of the RISK and SAFE pathways can be used for further drug development. Human studies are now underway to test some of these strategies, but further clinical trials are necessary to translate these therapies to clinical practice.

Keywords

cardiac arrest, cardiopulmonary resuscitation, post-conditioning, reperfusion injury, RISK pathway, SAFE pathway

Key Points

• Reperfusion injury is a primary contributor to the tissue damage and dysfunction following ischemic injury.

• Post-conditioning reduces reperfusion injury as demonstrated by improved cardiac function, improved neurological function, and improved survival in animal models of cardiac arrest.

• The exact mechanism of post-conditioning remains unclear but likely involves activation of the RISK and SAFE pathways in addition to beneficial effects of gradual normalization of pH and calcium levels. The result of these changes is reduced apoptosis and improved organ function.

• The benefits of post-conditioning have been well described in animal studies, but

extensive clinical trials will be necessary to understand the role of these treatments in humans.

INTRODUCTION

Survival after out-of-hospital cardiac arrest (OHCA) remains poor despite decades of innovation in prehospital and in-hospital care. Only 5–8% of patients survive to hospital discharge with good neurologic function (19–22). Reducing ischemic time with bystander cardiopulmonary resuscitation (CPR) and early defibrillation is associated with improved survival (23–26). However, additional tissue damage arises from restoration of blood flow into the ischemic territory, termed reperfusion injury (10,27). Reperfusion injury causes up to 50% of the total damage induced by myocardial infarction in animal models (10,11). Therapies initiated prior to ischemia are termed pre-conditioning. Therapies provided during ischemia but before reperfusion are called peri-conditioning. Treatment strategies initiated after reperfusion has begun are called post-conditioning. In the context of high-quality CPR, it is important to consider reperfusion to start at the onset of CPR rather than at return of spontaneous circulation (ROSC).

Post-conditioning is accomplished by targeting cellular signaling pathways which lead to cell death and tissue dysfunction. Many therapies can be utilized for both pre and post-conditioning, though in many cases, the cellular targets and mechanism of protection are not fully known (11,28). In the setting of OHCA, post-conditioning offers an important advantage over pre-conditioning. Postconditioning strategies allow therapy to begin when medical professionals arrive to the patient, often in the pre-hospital setting, with continuation in the hospital. This review will explore some of the most promising post-conditioning strategies currently in development to augment resuscitation efforts and improve outcomes after cardiac arrest.

MECHANISMS OF POST-CONDITIONING

Post-conditioning provides benefit by modulating innate cellular pathways thereby influencing the balance between pro-apoptotic and pro-survival signaling.

Mechanisms of cell injury

Apoptosis is the final common pathway for much of the detrimental signaling induced by reperfusion injury. Inhibition of apoptosis reduces infarct size, reduces inflammation, improves endothelial function and coronary blood flow, and improves cardiac function (29). Pro-apoptotic signals can be initiated by rapid changes in pH, intracellular calcium, reactive oxygen species, or ATP depletion. In addition, extrinsic pathways involving the inflammatory response with Fas and tumor necrosis factor (TNF) signaling can also initiate apoptosis (Fig. 1-2.1).

Mitochondria play a critical role in cardiac arrest and resuscitation given their central role in energy metabolism and apoptotic signaling cascades. The mitochondrial permeability transition pore (mPTP), a nonselective channel in the inner mitochondrial membrane, integrates multiple signaling pathways to determine the fate of the mitochondria and the cell at large (30–33). When open, mPTP collapses the mitochondrial membrane potential thereby uncoupling oxidative phosphorylation. The open mPTP also releases cytochrome c into the cytoplasm where it can promote apoptosis (34,35).

Reperfusion injury salvage kinase pathway

Two pro-survival pathways are currently understood in detail. The first pathway described for cellular protection was the reperfusion injury salvage kinase (RISK) pathway, which leads to activation of the PI3K-Akt and Erk signaling cascades (36–38). PI3K is activated by growth factor receptors and G-protein-coupled receptors providing for a diverse array of potential ligands or medications that may contribute to post-conditioning (39,40). Activation of PI3K also prevents calcium release from the sarcoplasmic reticulum via activation of the prosurvival protein, PKCe (41–43). Akt inhibits proapoptotic signaling proteins including caspases, Bax, Bad, and GSK-3b (44–47). Akt also activates endothelial nitric oxide synthase (eNOS) resulting in increased nitric oxide production (48). The subsequent activation of cGMP and PKG results in activation of sarco/endoplasmic reticulum calcium ATPase (SERCA) and increased calcium uptake into the sarcoplasmic reticulum thereby decreasing the overall calcium load affecting the mitochondria (49). Meanwhile, Erk activation also inhibits proapoptotic signaling and increases expression of prosurvival genes (50–52).

Survivor activating factor enhancement pathway

The survivor activating factor enhancement (SAFE) pathway promotes cell survival via TNF receptormediated activation of the JAK/STAT-3 pathway leading to stabilization of the mPTP in the closed state (53,54). When activated, STAT-3 also translocates to the nucleus where it induces transcription of multiple pro-survival genes including Bcl-2, growth factors, cell cycle regulators, and other transcription factors (55,56). STAT-3 activation results in inhibition of pro-apoptotic proteins including Bax (57), Bad (58), and GSK-3b (59). In addition to nuclear translocation, STAT-3 also localizes to the mitochondria suggesting a potential effect on the electron transport chain and thus the cell's energy production (60).

The RISK and SAFE pathways have been studied largely in isolation. However, some post-conditioning therapies have been shown to activate both pathways (e.g., ischemic post-conditioning, opioids, inhaled anesthetics, among others). There is also evidence that the two pathways interact, though the details are not fully known (59,61). Ultimately, the combination of these pro-survival pathways improves calcium homeostasis, reduces production of reactive oxygen species, increases production of nitric oxide, inhibits mPTP opening, and prevents apoptosis.

ISCHEMIC POST-CONDITIONING

Ischemic post-conditioning uses a series of brief ischemic episodes during the initial phase of reperfusion following an ischemic insult. It was the first form of post-conditioning developed, as it was adapted from previous work with ischemic preconditioning performed in 1986 (62,63). The benefit of gradual reperfusion after coronary occlusion was also first described in 1986 and was then confirmed a decade later resulting in reduced infarct size and improved endothelial function (64,65). Pre and post-conditioning have since been shown to provide similar benefits in animal studies and clinical trials in patients with myocardial infarction (11,66,67). Importantly, pauses in cerebral reperfusion following stroke have demonstrated improved neurologic function as well as reduced cerebral infarction, cerebral edema, and inflammatory markers (68,69,13,70). Liver, intestine, lung, and kidney function have also been shown to benefit from ischemic post-conditioning

(71,72,15,73).

Ischemic post-conditioning was only recently assessed in a porcine model of cardiac arrest including 15 min of untreated ventricular fibrillation (74–76). Ischemic post-conditioning, composed of four pauses of 20 s during the first 3 min of CPR, improved coronary and cerebral blood flow during CPR and after ROSC was achieved. Cardiac and neurologic functions were also improved when compared with standard CPR alone (75).

The protective mechanism of ischemic post-conditioning in cardiac arrest is not well understood. Gradual reperfusion may limit reperfusion injury by slowing the normalization of intracellular pH and calcium, thereby reducing production of reactive oxygen species, preventing opening of the mPTP, and preventing activation of the pro-apoptotic calcium-sensitive protease, calpain (77,78). In addition, ischemic post-conditioning activates the RISK and SAFE pathways likely via multiple signaling molecules including adenosine and TNF (11,54). Dopamine receptors have been shown to potentiate these pathways through PKC activation, though PKC stimulation is not required for ischemic post-conditioning (79). Together, these pathways may account for the marked benefits observed with ischemic post-conditioning. Large clinical trials will be necessary to understand the degree to which these benefits translate to humans.

PHARMACOLOGIC POST-CONDITIONING

Post-conditioning may occur through activation of the RISK and SAFE pathways or by reducing the rapid changes in intracellular environment that occur with reperfusion. The wide array of pathway activators provides various opportunities for pharmacologic manipulation.

Nitric oxide

Intracellular nitric oxide levels are increased with activation of the RISK pathway as a result of eNOS activation. This process is required for the beneficial effects of ischemic post-conditioning (80) and has been shown to prevent opening of the mPTP (81). Infusion of nitric oxide donors has been shown to prevent mPTP opening via a PKG-dependent mechanism in hepatocytes (82). In addition, nitric oxide is a potent vasodilator, which may impact CPR by improving cardiac output.

Sodium nitroprusside (SNP) is a nitric oxide donor and potent vasodilator currently used in clinical practice to dilate arterial vasculature and improve cardiac output in the setting of cardiogenic shock. SNP has now been tested in a porcine model of ventricular fibrillation arrest in combination with an enhanced CPR (eCPR) method, which directs blood toward the vital organs. The eCPR includes active compression-decompression CPR, an inspiratory impedance threshold device, and abdominal binding. SNP eCPR improves coronary perfusion pressure, carotid blood flow, arterial pH, and end-tidal CO2 when compared with eCPR alone (7,75,83–85). Rates of ROSC, cardiac function, neurologic recovery, and survival were also increased with SNP eCPR. Importantly, no improvement is observed when SNP is used in combination with standard CPR suggesting that mesenteric pooling and peripheral blood flow limit the effectiveness of SNP if eCPR is not performed. It is unclear to what degree SNP improves CPR by postconditioning versus improved hemodynamics during the resuscitation. Further studies examining the post-conditioning pathways are necessary to understand the relative contribution of the RISK and SAFE pathways. Clinical trials will also be necessary to assess the effects of sodium nitroprusside in humans with OHCA.

Nitrite is a precursor for nitric oxide that undergoes conversion to nitric oxide in the setting of low oxygen and low pH leading to preferential conversion in areas of ischemia. Nitrite has shown improvement in cardiac function, neurologic function, and survival in rodent models of cardiac arrest (86,87). The benefits were attributed to reversible inhibition of mitochondrial complex I, which reduced the production of reactive oxygen species. No hemodynamic changes were observed. Activation of the RISK and SAFE pathways were not evaluated in these studies. Phase I clinical trials have demonstrated safety in humans (87) and further clinical trials are underway to assess its use in cardiac arrest and acute myocardial infarction (NCT01178359, NCT00924118).

Adenosine

Adenosine is an arteriolar vasodilator with additional effects including increased endothelial and myocardial ATP stores, inhibition of platelet aggregation, and inhibition of neutrophils leading to reduced levels of reactive oxygen species. In addition, adenosine has been implicated as a critical activator of the RISK pathway. Ischemic post-conditioning

and activation of the RISK pathway was eliminated in rodents lacking the adenosine receptors A2a (88) or A2b (89) confirming the importance of adenosine signaling in post-conditioning.

Canine models of myocardial infarction were the first used to assess the benefits of intracoronary adenosine showing reduced infarct size, increased epicardial and endocardial blood flow after reperfusion, and improved vasodilatory reserve in animals treated with adenosine (90,91). Reduced endothelial damage and improved microcirculation were implicated as the causes of the benefit. Importantly, these benefits did not occur if reperfusion began more than 3h after coronary occlusion suggesting that progression of the infarct during that time made adenosine ineffective (92).

Adenosine has also been tested in humans with ST-segment elevation myocardial infarction (STEMI) demonstrating reduced infarct size, improved survival, and reduction in a composite endpoint including new heart failure, re-hospitalization for heart failure, or death from any cause within 6 months (93,94). These effects were only observed in patients who underwent reperfusion therapy within 3h of symptom onset emphasizing the importance of early administration similar to the canine studies.

Studies of adenosine in CPR are limited. Adenosine was tested in a porcine model of ventricular fibrillation arrest in combination with SNP eCPR (76). Adenosine-treated animals demonstrated a further increase in cerebral blood flow and cardiac function beyond that achieved with SNP eCPR alone. The activation of RISK or SAFE pathways was not assessed. Further animal and human studies will be necessary to examine the role of adenosine in post-conditioning after cardiac arrest.

Cyclosporine

Cyclosporine A prevents opening of the mPTP by inhibiting the calcium-dependent interaction of cyclophilin D with the pore. This effect mimics activation of the RISK and SAFE pathways, but bypasses the signaling pathways altogether. Studies using porcine models of cardiac arrest have shown improved mitochondrial integrity and respiration, reduced apoptosis, improved cardiac function, and improved survival with use of cyclosporine early in the resuscitation (95–97). Delayed administration by as little as 3 min after ROSC eliminated the benefit in a rat model of cardiac arrest (96,98). Previous clinical

trials have demonstrated a reduction in infarct size with cyclosporine treatment in patients with a STEMI (35,99). A clinical trial is currently underway to examine the potential benefit of cyclosporine therapy during CPR in humans (NCT01595958).

Opioids

Opioids induce post-conditioning in animal models via activation of both the RISK and SAFE pathways by ∂ opioid receptors (59,100–103). Several studies have shown reduced infarct size and reduced apoptosis using remifentanil, morphine, and sufentanil in rat models (100,104–106). No human trials have confirmed these results, but opioids are commonly used for sedation in the setting of cardiac arrest, myocardial infarction, and stroke.

Inhaled anesthetics

In the setting of cardiac arrest, inhaled anesthetics provide the opportunity to deliver medication at the initiation of resuscitation via a face mask thereby maximizing the benefits of post-conditioning.

Sevoflurane is a halogenated anesthetic used extensively for surgical patients. The cardiac effects of halogenated anesthetics have been studied extensively (107); however, the mechanism of the cardioprotective effect remains unclear. Sevoflurane and isoflurane have been shown to activate the RISK pathway in cardiomyocytes and neurons, but the mechanism of activation is unknown (108–110). Preconditioning with sevoflurane has been shown to reduce cerebral infarct size, preserve mitochondrial function, reduce ROS, and improve long-term neurological function in a rat model of stroke (111). Sevoflurane also reduces cardiac apoptosis and improves cardiac function in porcine models of cardiac arrest (112). Further study is necessary to assess the role for sevoflurane in improving neurological outcomes.

Xenon is a noble gas, which is known to inhibit N -methyl-D -aspartate (NMDA) receptors in the central nervous system. Inhibition of NMDA receptors may reduce calcium influx and activation of the mPTP, which may prevent the neuronal death induced by cardiac arrest and stroke. Improvements in neuronal necrosis and perivascular inflammation were observed in a porcine model of cardiac arrest when xenon was delivered after ROSC was achieved (113). Long-term improvements in neurological function were

not observed.

Na^+/H^+ exchanger inhibitors

Cariporide is a selective inhibitor of the sarcolemmal Na^+/H^+ exchanger (NHE). The NHE can cause excessive intracellular sodium levels during reperfusion as the cell corrects intracellular acidosis. Sodium overload can induce excessive calcium release from the sarcoplasmic reticulum through the Na^+ /Ca^{2+} exchange pump. Elevated cytosolic calcium levels lead to mitochondrial destruction, apoptosis, and arrhythmias. Cariporide treatment reduced ventricular ectopy and the need for postresuscitation defibrillation in a porcine model of cardiac arrest (114,115). Cardiac function and mean arterial blood pressure were also improved, whereas neurological outcomes were similar between cariporide treatment and placebo. Sabiporide is a longacting NHE inhibitor, which has also been shown to improve cardiac function and oxygen delivery to vital organs (116). It also showed an overall reduction in the systemic inflammatory response after cardiac arrest.

CONCLUSION

Substantial effort has been invested in developing and understanding post-conditioning strategies for cardiac arrest. The various medications known to induce post-conditioning converge to achieve their therapeutic effects via a small number of pro-survival signaling pathways. Challenges arise when testing the mechanisms of these agents, as many of the medications have substantial hemodynamic effects in addition to any role in postconditioning. For example, the vasodilation induced by SNP may improve cardiac output and clinical outcomes. Similarly, comorbidities such as age, diabetes, hypertension, and hyperlipidemia have been shown to impact the fidelity of these post-conditioning pathways, though the degree of this impact is unknown (110,117). These factors will be important when designing clinical trials as well as therapeutic strategies for individual patients. Although there have been small human studies in the past, and future studies are planned or underway, it has yet to be seen if these results in animals will translate to the clinical environment.

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Conflicts of interest

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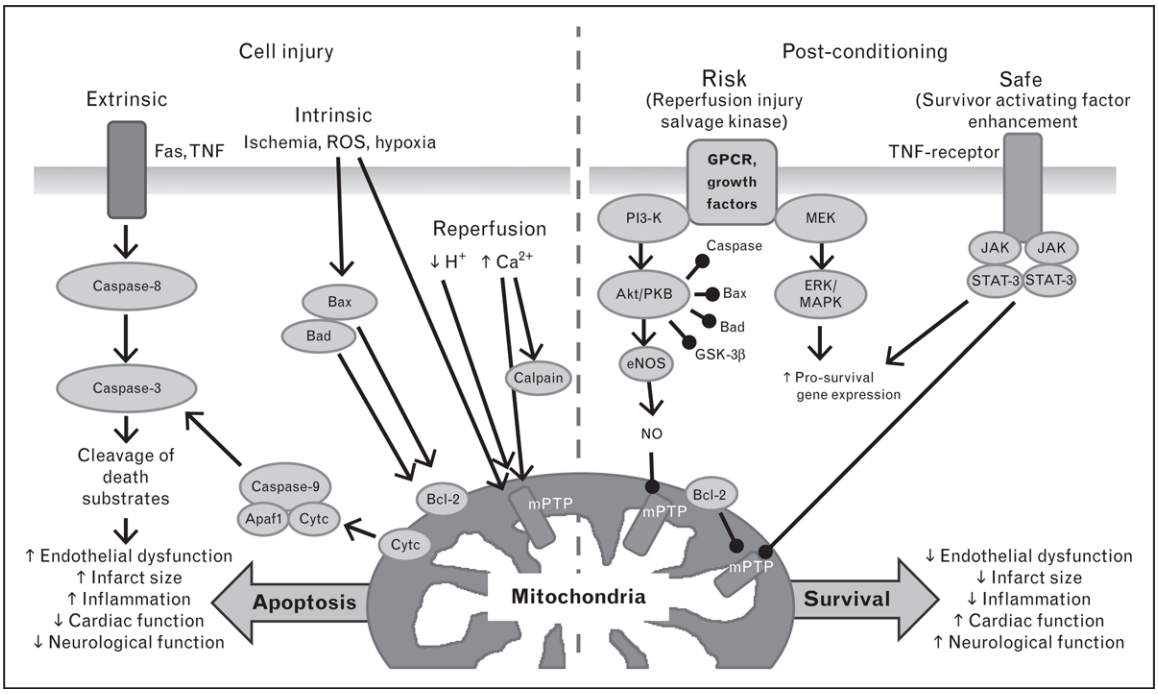

Figure 1.1. Cell injury and post-conditioning pathways active during ischemia-reperfusion. The mechanisms of cell injury (Left) include extrinsic, intrinsic, and reperfusion injuries. The post-conditioning pathways (Right) include the RISK and SAFE pathways. Many pathways lead to the mitochondria, which integrates the signals to balance the pro apoptotic and pro-survival signals. Activation of Erk and STAT-3 also results in signal transduction to the nucleus where expression of pro-survival genes is increased. The apoptotic and survival pathways result in opposing physiologic and clinical outcomes as noted. Arrows indicate activation whereas rounded lines indicate inhibition. GPCR, G-protein coupled receptor; mPTP, mitochondrial permeability transition pore; NO, nitric oxide; RISK, reperfusion injury salvage kinase; ROS, reactive oxygen species; SAFE, survivor activating factor enhancement; TNF, tumor necrosis factor.

CHAPTER 2

Ischemic postconditioning at the initiation of cardiopulmonary resuscitation facilitates functional cardiac and cerebral recovery after prolonged untreated ventricular fibrillation

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Synopsis

Objectives: Ischemic postconditioning (PC) with "stuttering" reintroduction of blood flow after prolonged ischemia has been shown to offer protection from ischemia reperfusion injury to the myocardium and brain. We hypothesized that four 20-s pauses during the first 3 min of standard CPR would improve post resuscitation cardiac and neurological function, in a porcine model of prolonged untreated cardiac arrest.

Methods: 18 female farm pigs, intubated and isoflurane anesthetized had 15 min of untreated ventricular fibrillation followed by standard CPR (SCPR). Nine animals were randomized to receive PC with four, controlled, 20-s pauses, during the first 3 min of CPR (SCPR + PC). Resuscitated animals had echocardiographic evaluation of their ejection fraction after 1 and 4 h and a blinded neurological assessment with a cerebral performance category (CPC) score assigned at 24 and 48 h. All animals received 12 h of post resuscitation mild therapeutic hypothermia.

Results: SCPR + PC animals had significant improvement in left ventricular ejection fraction at 1 and 4 h compared to SCPR (59 ± 11% vs 35 ± 7% and 55 ± 8% vs 31 ± 13% respectively, $p < 0.01$). Neurological function at 24 h significantly improved with SCPR + PC compared to SCPR alone (CPC: 2.7 ± 0.4 vs 3.8 ± 0.4 respectively, $p = 0.003$). Neurological function significantly improved in the SCPR + PC group at 48 h and the mean CPC score of that group decreased from 2.7 ± 0.4 to 1.7 ± 0.4 ($p < 0.00001$).

Conclusions: Ischemic postconditioning with four 20-s pauses during the first 3 min of SCPR improved post resuscitation cardiac function and facilitated neurological recovery after 15 min of untreated cardiac arrest in pigs.

1. Introduction

Cardiopulmonary resuscitation (CPR) survival rates have remained poor over the past half-century with only minimal if any improvements in neurologically intact survival (118). In humans, when ventricular fibrillation (VF) is left untreated for more than 10 min, short and long term survival is severely reduced (119). In animals, current non-invasive methods of CPR are unable to provide short and long-term neurological recovery when ventricular fibrillation is left untreated longer than 12–13 min (120–122). Furthermore, successful resuscitation following 12–15 min of untreated VF has been used as the model to evaluate post resuscitation left ventricular dysfunction (123).

Re-introduction of blood flow with "controlled pauses" has been shown to protect the myocardium and the brain from ischemia-reperfusion injury in clinical scenarios of regional ischemia during ST elevation myocardial infarction and stroke both in animals and humans (11,30,13,124). This concept has been called "ischemic postconditioning" and describes a method of reperfusion injury protection (10,11).

We sought to introduce ischemic postconditioning at the initiation of CPR efforts by providing four, 20-s pauses in chest compressions and ventilations over the first 3 min of reperfusion. We hypothesize that the implementation of a simple ischemic postconditioning (PC) strategy during the first 3 min of CPR, will improve cardiac and cerebral function and 48-h survival rates after 15 min of untreated ventricular fibrillation.

2. Materials and methods

All studies were performed by an experienced research team in Yorkshire female farm pigs weighing 32 ± 2 kg. A certified and licensed veterinarian provided a blinded neurological assessment at 24 and 48 h. The protocol was approved by the Institutional Animal Care Committee of the Minneapolis Medical Research Foundation of Hennepin County Medical Center. All animal care was compliant with the National Research Council's 1996 Guidelines for the Care and Use of Laboratory Animals.

2.1. Preparatory phase

The anesthesia, surgical preparation, data monitoring, and recording procedures used in this study have been described previously (125). Briefly, we employed aseptic surgical conditions using initial sedation with intramuscular ketamine (7 mL of 100 mg/mL,

Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa) followed by inhaled isoflurane at a dose of 0.8–1.2%. Pigs were intubated with a size 7.0 endotracheal tube. The animal's temperature was maintained at 37.5 ± 0.5 ˚C, with a warming blanket (Bair Hugger, Augustine Medical, Eden Prairie, Minnesota). Central aortic blood pressure was recorded continuously with a micromanometer-tipped (Mikro-Tip Transducer, Millar Instruments, Houston, Texas) catheter placed at the beginning of the descending thoracic aorta. A second Millar catheter was inserted in the right atrium via the right external jugular vein. All animals received an intravenous heparin bolus (100 units/kg) and 500 units of heparin every hour until surgical repair was completed. An ultrasound flow probe (Transonic 420 series multichannel, Transonic Systems, Ithaca, New York) was placed to the right internal carotid artery to record blood flow (mL/min). The animals were then ventilated with room air, using a volume-control ventilator (Narcomed, Telford, Pennsylvania), with a tidal volume of 10 mL/kg and a respiratory rate adjusted to continually maintain a $PaCO_2$ of 40 mmHg and PaO_2 of 80 mmHg (blood oxygen saturation > 95%), as measured from arterial blood (Gem 3000, Instrumentation Laboratory, Lexington, Massachusetts) to adjust the ventilator as needed. Surface electrocardiographic tracings were continuously recorded. All data were recorded with a digital recording system (BIOPAC MP 150, BIOPAC Systems, Inc., CA, USA). End tidal CO_2 ($ETCO_2$), tidal volume, minute ventilation, and blood oxygen saturation were continuously measured with a respiratory monitor (CO_2SMO Plus, Novametrix Medical Systems, Wallingford, Connecticut).

2.2. Measurements and recording

Thoracic aortic pressure, right atrial pressure, $ETCO_2$, and carotid blood flow were continuously recorded. Coronary perfusion pressure (CPP) during CPR was calculated from the mean arithmetic difference between right-atrial pressure and aortic pressure during the decompression phase. Carotid artery blood flow was reported in mL/s.

2.3. Experimental protocol

After the surgical preparation was complete, oxygen saturation on room air was greater than 95%, and $ETCO_2$ was stable between 35 and 42 mmHg for 5 min, ventricular fibrillation was induced by delivering direct intracardiac current via a temporary pacing wire (St. Jude Medical, Minnetonka, Minnesota). The ventilator was disconnected from the

endotracheal tube. Standard CPR was performed with a pneumatically driven automatic piston device (Pneumatic Compression Controller, Ambu International, Glostrup, Denmark) as previously described (126). During S-CPR, uninterrupted chest compressions were performed at a rate of 100 compressions/ min, with a 50% duty cycle and a compression depth of 25% of the anterior–posterior chest diameter. Asynchronous positive-pressure ventilations were delivered with room air (FiO_2 of 0.21) with a manual resuscitator bag. The tidal volume was maintained at ~10 mL/kg and the respiratory rate was 10 breaths/min. The investigators were blinded to hemodynamics during CPR.

2.4. Protocol

Following 15 min of untreated ventricular fibrillation, 18 pigs were randomized to receive standard CPR (SCPR) or SCPR plus postconditioning (SCPR + PC). The SCPR + PC group received initially 40 s of SCPR followed by a 20-s pause of compressions and ventilations followed by another 20 s of SCPR and the cycle was repeated for a total of 4 pauses (Fig. 2.1). Epinephrine was administered in both groups in 0.5 mg (~15 mcg/kg) bolus at minute three and was repeated every 3 min until return of spontaneous circulation (ROSC). Resuscitation efforts were continued until ROSC was achieved or a total of 15 min of CPR had occurred. The first defibrillation effort was delivered with 200-J biphasic shocks after 4 min of CPR in both groups. If ROSC was not achieved, defibrillation was delivered every 2 min thereafter during CPR.

2.5. Post ROSC care

After ROSC was achieved, animals were connected to the mechanical ventilator. Supplemental oxygen was added only if arterial saturation was lower than 90%. Animals were observed under general anesthesia with isoflurane until hemodynamically stable. Hemodynamic stability was defined as a mean aortic pressure > 55 mmHg without pharmacological support for 30 min and normalization of $ETCO_2$ and acidosis. Animals that had a stable post-ROSC rhythm but were hypotensive (mean arterial pressure < 50 mmHg) received increments of 0.1–0.2 mg intravenous epinephrine every 5 min until MAP rose above 50 mmHg. If pH was lower than 7.2, 100 mEq of $NaHCO_3$ was given intravenously. Amiodarone 20 mg intravenously was given in all animals if there was recurrence of ventricular fibrillation after initial ROSC. At that point vascular repair of the

internal jugular vein and the left common femoral artery were then performed. Arterial blood gases were obtained at baseline, at 5 min after ROSC and every 30 min thereafter.

Both groups received post resuscitation therapeutic hypothermia as per American Heart Association recommendations for resuscitated comatose patients from ventricular fibrillation in order to simulate best practice and optimize the chances of the control group for neurological recovery. Our institutional animal care committee mandated therapeutic hypothermia for protocol approval. Immediately after ROSC all animals in both groups received 1.5 l of chilled Saline (8–10 ˚C) and surface cooling with towels soaked with ethanol. Target temperature was set at 34 ˚C and was maintained at that level for 12 h with the use of a cooling blanket device (Arctic sun, Medivance Inc., Louisville, CO, USA). Target temperature was reached within 29 ± 8 and 31 ± 6 min in the control and IPC groups respectively ($p = 0.56$). Central temperature was measured at the bladder of the animals. Animals were re-warmed at 0.5 ˚C/h to 36 ˚C. Subsequently, animals were weaned off anesthesia and were extubated once they could maintain normal respiratory pattern and blood gasses for 30 min off the ventilator.

Survivors were given intramuscular analgesic injections of nonsteroidal anti-inflammatory medication as previously described and had free access to water and food (7). No other post-ROSC medical care was provided after the vascular repair. Animals were returned to their runs and were observed every 2 h for the next 6 h for signs of distress or accelerated deterioration of their function. If animals met predetermined criteria or if the veterinarian judged that they were in severe distress they were euthanized per IACUC protocol.

2.6. Neurological assessment

Twenty-four and 48-h after ROSC, a certified veterinarian, blinded to the intervention, assessed the pigs' neurological function based upon a cerebral performance category (CPC) scoring system modified for pigs. The veterinarian used clinical signs such as response to opening the cage door, response to noxious stimuli if unresponsive, response to trying to lift the pig, whether the animal could stand, move all four limbs, walk, eat, urinate, defecate, and respond appropriately to the presence of a person walking into the cage. The following scoring system was used: 1 = normal; 2 = slightly disabled; 3 =

moderately disabled but conscious; 4 = vegetative state.14 For each group, mean CPC score (excluding deaths) was calculated at 24 and 48 h

2.7. Echocardiographic evaluation of left ventricular function

A transthoracic echocardiogram was obtained on all survivors 1 and 4 h post ROSC. Images were obtained from the right parasternal window that provides similar views as the long and short parasternal windows in humans (127). Ejection fraction was assessed using Simpson's method of volumetric analysis by an independent clinical echocardiographer blinded to the treatments (128). Before echocardiographic evaluation, any inotropic support was stopped for at least 20 min and, if needed, was restarted immediately after the echocardiographic evaluation.

2.8. Statistical analysis

Values were expressed as mean ± standard deviation. Baseline data, hemodynamic parameters, blood gases and left ventricular ejection fraction measurements between groups were analyzed with unpaired t-test. A 2-tailed Fischer exact test was used to compare 24 and 48-h survival rate. Unpaired t-test was used to evaluate mean CPC scores between the two groups at 24 h. A paired t-test was used for comparison between 24 and 48-h mean CPC score of the SCPR + PC. The primary study endpoint was the mean CPC score at 24 h and left ventricular ejection fraction at 4 h. Secondary endpoint was survival at 48 h after ROSC. A p-value of <0.05 was considered statistically significant.

3. Results

There were no significant baseline differences between treatment groups in any hemodynamic or respiratory parameters (Tables 2.1 and 2.2).

At the end of 15 min of untreated fibrillation before CPR initiation, the core body temperature of the animals was 36.9 ± 0.3 ˚C and 37.1 ± 0.2 ˚C in the SCPR and SCPR + PC groups respectively.

3.1. CPR hemodynamics

Both groups had similar aortic and right atrial pressures with similar pre epinephrine coronary perfusion pressures. The SCPR + PC group demonstrated significantly higher post epinephrine aortic and coronary perfusion pressures compared to SCPR alone (Table

2.1).

3.2. Return of spontaneous circulation and survival

There were no significant differences in ROSC and 24 h survival between groups (Table 2.1). In the S-CPR group, 8/9 animals achieved ROSC, and 5/9 animals survived 24 h. Only 1/9 animal survived to 48 h. In the SCPR + PC group, 9/9 animal had initial ROSC and 8/9 survived to 24 and 48 h ($p = 0.003$ for 48 h survival rate). Animals in the SCPR + PC group were significantly more stable and received significantly less epinephrine than the control animals during the recovery period (Table 2.1). Three of the five animals treated with SCPR that had ROSC, died during the first night. Animals that had a CPC score of 4 (coma) at 24 h died before the 48 h evaluation.

3.3. Left ventricular function

Echocardiographic evaluation at 1 h revealed that animals receiving SCPR alone had a significantly lower left ventricular ejection fraction than the animals treated with SCPR + PC who appeared to have normal function ($35 \pm 7\%$, vs $59 \pm 11\%$, $p < 0.01$). The effect was maintained at 4 h ($31 \pm 13\%$ vs $55 \pm 8\%$, $p < 0.01$) (Table 2.1).

3.4. Neurological function at 24 and 48 h

Mean CPC score at 24 h was significantly lower (better neurological function) in the animals that received SCPR + PC compared to SCPR alone (2.7 ± 0.4 vs 3.8 ± 0.4 respectively, $p = 0.003$). Neurological function in SCPR + PC group significantly improved in all but one animals at 48 h and the mean CPC score of the group decreased from 2.7 ± 0.4 to 1.7 ± 0.4 ($p < 0.001$). The one SCPR animal that survived 48 h had the same CPC score of 3 at 24 and 48 h (Fig. 2.2).

3.5. Blood gasses, end tidal CO2 and lactate

There were no significant differences in blood gas values at baseline between groups. Immediately after ROSC, pH and HCO_3 and $ETCO_2$ were significantly higher in the SCPR + PC group a finding that can be explained by higher circulation at the last few minutes of CPR and immediately after ROSC (Table 2.2).

4. Discussion

Our study, for the first time, shows that a simple strategy of ischemic postconditioning introduced early during standard-CPR with four 20-s pauses can significantly improve cardio-cerebral outcomes in a porcine model of very prolonged cardiac arrest and global ischemia. When good quality SCPR was coupled with controlled pauses at the initiation of reperfusion, the resuscitated animals documented normal left ventricular function post resuscitation in the absence of inotropic support and improved neurologic outcome. Furthermore, to our knowledge, this is the only study that has demonstrated complete neurological recovery is possible after 15 min of untreated cardiac arrest with standard CPR and a non-invasive intervention.

It is important to emphasize that unintentional pauses in chest compressions spread throughout resuscitative efforts have been associated with worse outcomes by adding to the injury that has accumulated from the no-flow period (129–131). The effects of pauses on coronary perfusion pressure and carotid blood flow were exactly as previously described by Berg et al (130). Pauses caused elimination of the trans-coronary pressure gradient and carotid flow. The type of intentional pauses described in this report is thought to harness endogenous protective processes associated with specific mitochondrial protective mechanics and should not be confused with the poor outcomes known to be associated with poor CPR quality that includes prolonged intervals of interrupted chest compressions (132).

The fact that there were no differences in ROSC rates between groups and that the S-CPR (control) group had such high ROSC rate is a testament to the high quality of CPR performed. While introduction of CPR pauses at the initiation of resuscitation efforts may appear to be contrary to some beliefs that continuous uninterrupted chest compressions are essential, four pauses of 20 s duration during the first 3 min after CPR initiation followed by continuous chest compressions with asynchronous ventilations for the remainder of the resuscitation appeared to positively impact neurological outcome after very prolonged global cerebral ischemia (133).

The animals that were treated with controlled pauses showed absence of post resuscitation left ventricular dysfunction and they were hemodynamically more stable post

ROSC requiring less epinephrine. There is very strong evidence that post conditioning is advantageous for cardiac muscle protection after ischemia (10,134). Three to four pauses during reperfusion of acute myocardial infarction have been shown to significantly decrease infarct size in human studies (30,124). Our results show that a similar strategy of controlled reperfusion after prolonged global ischemia in cardiac arrest exhibits the same benefits for the myocardium as a whole and mitigates post ROSC cardiac dysfunction that contributes heavily to post resuscitation morbidity and mortality. Recently animal studies have described strategies to alleviate ischemia reperfusion injury and promote cardiac recovery after cardiac arrest with inhaled anesthetics and hypothermia. It is possible that a strategy combining pauses during CPR and pharmacological postconditioning could demonstrate further benefits (112,135).

The most striking observation in our study was that the brain demonstrated the potential for full recovery after 15 min of global ischemia with no flow. Shaffner et al. showed that cerebral recovery was not feasible after 12 min of untreated arrest because regeneration of ATP was not possible despite high cerebral perfusion pressures (136). Our data suggest otherwise. To our knowledge, this is the first time that survival rates with consistently favorable neurological outcomes have been reported after 15 min of untreated cardiac arrest. Controlled reperfusion with short pauses at the initiation of reperfusion of stroke has been shown to significantly decrease injury in a rat model (13). Furthermore, 15–30 s cycles of on/off flow in the same model with 10 min of global ischemic cerebral insult have provided significant cerebral preservation and recovery (13). The latter model is relevant to cardiac arrest where the ischemic insult is systemic and global. For the above mentioned reasons we combined the duration (15–30 s) and number of cycles (3–4) of different ischemic postconditioning strategies to create our tested protocol of four 20-s pauses during the first 3 min of CPR.

The mechanisms for protection of both the heart and brain have been well-studied and are currently considered to be mediated by direct and indirect modulation of mitochondrial permeability transition pore state (132,137–141). We used this strategy exactly because of previously documented protection of vital organs in stroke and myocardial infarction. Based on our data, it appears that postconditioning with pauses in

CPR efforts at the initiation of reperfusion, offer significant protection and facilitates functional cardiac and cerebral recovery (10,30,142,143).

Postconditioning with controlled reperfusion has also been shown to be beneficial in preventing ischemia reperfusion injury in most of the organs such as liver, kidney, retina, and small intestine (144–150). That could explain why the animals with SCPR + CP had an overall improvement in their status, survived and continued to improve to 48 h.

This study has limitations. First, we did not perform a dosing study and therefore we cannot comment what is the best combination of cycles and duration of pauses. We used the specific combination based on the literature targeting neurological protection (13). Second we do not know if the same benefits could be realized if resuscitation efforts are prolonged and we cannot exclude the possibility of synergy between mild therapeutic hypothermia and ischemic postconditioning. Third, our study was not designed to address the mechanism of protection offered by postconditioning. Nonetheless, we have no reason to believe that the mechanism should be different than the one described extensively in the cardiac and cerebral literature (10,30,142,143). We also did not assess biomarkers of injury for the heart and brain. Although the clinical endpoints reported here (echocardiographic LV function and blinded neurological assessment), in our opinion, represent higher quality preclinical endpoints than biomarkers, we cannot claim there was tissue protection despite the observed improvement in myocardial and cerebral functional outcomes. A histopathology/brain MRI study is underway to correlate the clinical endpoints observed in this study with brain pathology. However, we tested those interventions exactly as described for acute myocardial infarction and stroke and found them to be extremely effective in our model of cardiac arrest (30,142,143). Finally, it is unknown if the benefits demonstrated in this study would be seen with coexisting myocardial ischemia or can be translated to humans.

5. Conclusion

A simple strategy of ischemic postconditioning with four 20-s pauses during the first 3 min of SCPR mitigates post resuscitation cardiac dysfunction and facilitates neurological recovery after 15 min of untreated cardiac arrest in pigs.

Authors' note

All authors have participated to the conception, design and writing of this manuscript.

Conflict of interest

Demetris Yannopoulos is the Medical Director of the Minnesota Resuscitation Consortium, a state wide initiative to improve survival in the state of MN from cardiac arrest. This initiative is sponsored by the Medtronic Foundation and is part of the Heart Rescue Program. There are no conflicts related to this investigation.

Keith G. Lurie is the founder of Advanced Circulatory Systems Incorporated (ACSI), and co-inventor of the inspiratory impedance threshold device and ACD CPR technique but he has no conflicts to this study.

Tom P. Aufderheide has board membership for Take Heart America and Citizen CPR Foundation, has consulted for JoLife Medical and Medtronic Foundation, and has received grants/grants pending from the NHLBI Immediate Trial, NHLBI Resuscitation Outcomes Consortium and NINDS Neurological Emergency Treatment Trials Network.

Henry R Halperin MD is a consultant to Zoll Medical and has received grants from NHLBI.

Menekhem Zviman is a consultant to Zoll Medical.

The other authors have no conflicts of interest with the present study.

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Ethical approval

The study was approved by the Institutional Animal Care Committee of the Minneapolis Medical Research Foundation of Hennepin County Medical Center, and all animals received treatment in compliance with the National Research Council's 1996 Guide for the Care and Use of Laboratory Animals.

CPR method	Measurement	Baseline	2 min CPR	4 min CPR (after 0.5 mg EPI)	1 h ROSC	4 h ROSC	Number of shocks to initial ROSC	Total EPI dose (mg)	ROSC	24 h survival	48 h survival
SCPR + PC	SBP	94 ± 11	60 ± 11	112 ± 10*	95 ± 5	83 ± 4	2.7 ± 2	0.8 ± 0.3*	9/9	8/9	8/9*
	DBP	67 ± 8	31 ± 5	53 ± 4*	59 ± 4	57 ± 4					
	RA	2 ± 1	4 ± 0	4 ± 1	6 ± 1	3 ± 3					
	CPP	75 ± 9	27 ± 5	49 ± 7*	67 ± 5	55 ± 4					
	CBF	184 ± 45	47 ± 7	38 ± 4*	217 ± 24	181 ± 37					
	LVEF %	64 ± 6	N/A	N/A	59 ± 11*	55 ± 8*					
SCPR	SBP	91 ± 8	66 ± 8	88 ± 16	91 ± 7	88 ± 8	3 ± 4	1.5 ± 0.5	8/9	5/9	1/9
	DBP	62 ± 9	20 ± 6	41 ± 8	56 ± 9	61 ± 9					
	RA	3 ± 2	3 ± 1	2 ± 3	0.5 ± 2	4 ± 2					
	CPP	59 ± 4	26 ± 4	39 ± 6	56 ± 8	57 ± 4					
	CBF	181 ± 41	39 ± 15	18 ± 7	167 ± 46	172 ± 33					
	LVEF %	66 ± 5	N/A	N/A	35 ± 7	31 ± 13					

Table 2.1. Hemodynamics, resuscitation rates. Values are shown as mean ± SD. CPR was performed with either SCPR or SCPR + PC. All pressures in mmHg, all flows in mL/min. SBP = systolic blood pressure, DBP = diastolic blood pressure, RA = right atrial pressure, CPP = coronary perfusion pressure, CBF = carotid blood flow, LVEF: left ventricular ejection fraction (%).

* Mean statistically significant difference between groups with $p < 0.05$ SCPR: standard CPR; PC: postconditioning.

Arterial blood gasses		Baseline	5 min ROSC	30 min ROSC	120 min ROSC
SCPR+PC	pH	7.45 ± 0.04	7.26 ± 0.08*	7.35 ± 0.03	7.42 ± 0.05
	pCO_2	40 ± 5	47 ± 3*	38 ± 4	40 ± 4
	pO_2	90 ± 9	90 ± 13	84 ± 5	92 ± 11
	HCO_3	23.4 ± 0.5	21 ± 3*	21 ± 2	25 ± 2*
	$ETCO_2$	38 ± 3	44 ± 7*	37 ± 2	33 ± 3
SCPR	pH	7.42 ± 0.02	7.16 ± 0.04	7.34 ± 0.02	7.38 ± 0.06
	pCO_2	41 ± 6	38 ± 1	37 ± 2	35 ± 6
	pO_2	94 ± 7	85 ± 11	90 ± 5	92 ± 6
	HCO_3	29.3 ± 0.6	15 ± 2	19 ± 3	18.1 ± 0.7
	$ETCO_2$	41 ± 1	33 ± 6	38 ± 1	35 ± 2

Table 2.2. Arterial blood gasses during cardiopulmonary resuscitation and after return of spontaneous circulation. Mean ± SD. Arterial blood gas measurements at baseline and after ROSC. Partial pressures in torr. SaO_2: percent oxygen saturation; HCO_3: bicarbonate; $ETCO_2$: end-tidal CO_2.

* Means statistically significant difference between groups.

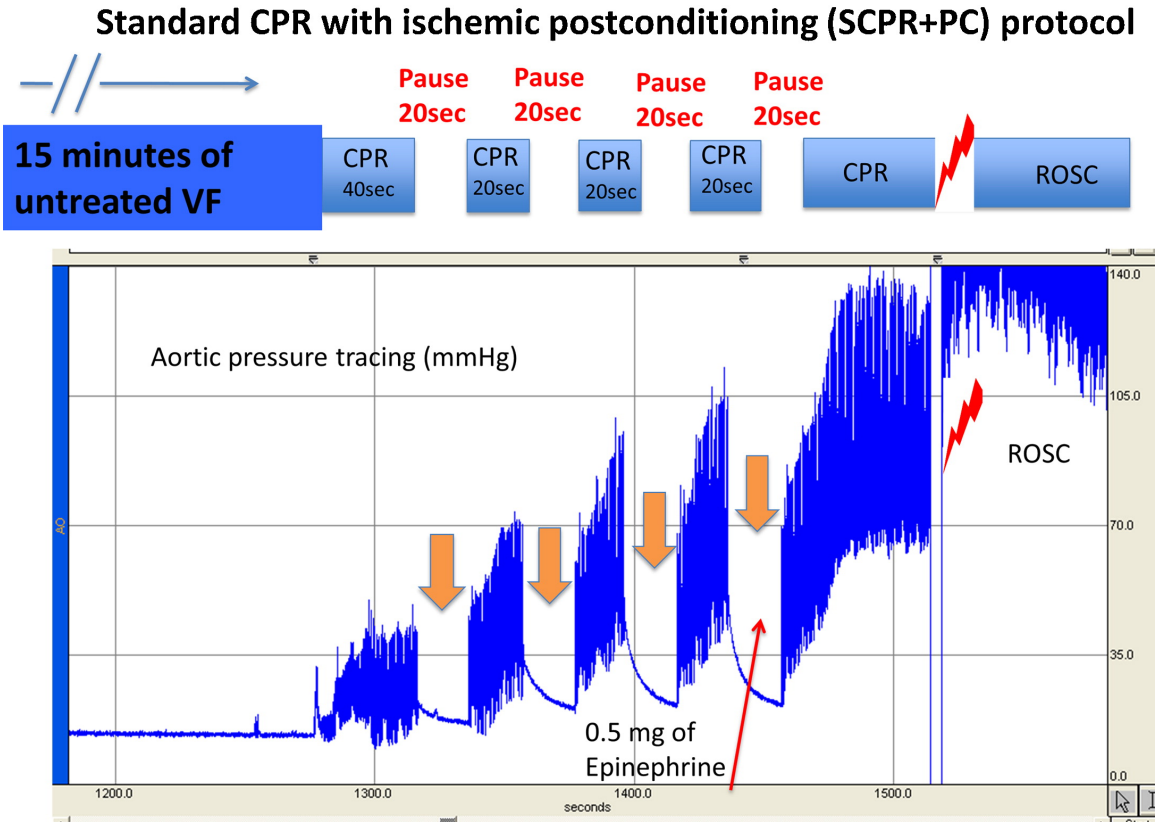

Figure 2.1. Ischemic postconditioning protocol during standard cardiopulmonary resuscitation. In the SCPR + PC group during the first 3 min of CPR, animals received four 20-s pauses and each pause was followed by 20 s of SCPR. The "stuttering" introduction of reperfusion is called "ischemic postconditioning". SCPR: standard CPR; VF: ventricular fibrillation; ROSC: return of spontaneous circulation.

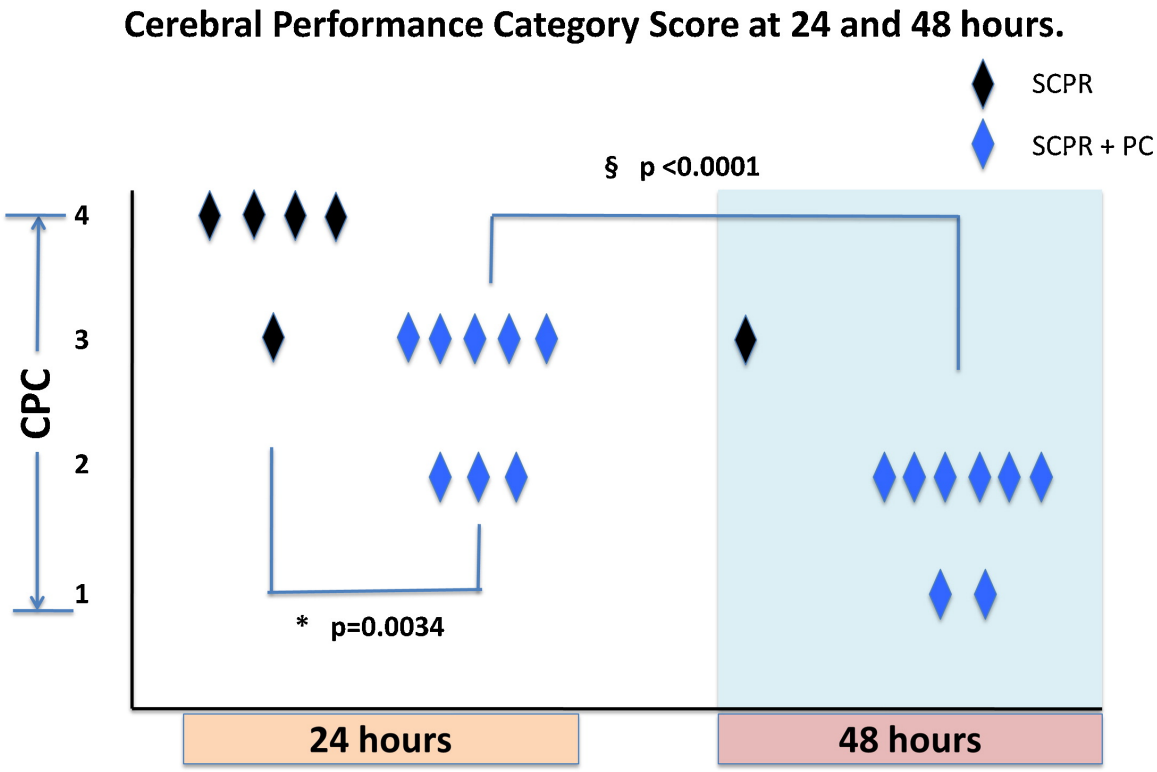

Figure 2.2. 24 and 48-h neurological assessment. Addition of controlled pauses during SCPR significantly improved neurological function compared to SCPR alone at 24 h. In the SCPR + PC neurological function improved in all but one animal at 48 h. Mean CPC at 24 h was significant lower in the SCPR + PC compared to SCPR alone (CPC: 2.7 ± 0.4 vs 3.8 ± 0.4 respectively, p = 0.003) cerebral performance category score: (1 = normal, 2 = mild deficit, 3 = moderate deficit but conscious, 4 = coma); SCPR: standard CPR. PC: postconditioning. [*]Means statistically significant difference between groups at 24 h. [§]Means statistically significant difference between the SCPR + PC group at 24 vs 48 h.

CHAPTER 3
PART 1

Ischemic post-conditioning and vasodilator therapy during standard cardiopulmonary resuscitation to reduce cardiac and brain injury after prolonged untreated ventricular fibrillation

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Synopsis

Aim of the study: We investigated the effects of ischemic postconditioning (IPC) with and without cardioprotective vasodilatory therapy (CVT) at the initiation of cardiopulmonary resuscitation (CPR) on cardio-cerebral function and 48-hour survival.

Methods: Prospective randomized animal study. Following 15 min of ventricular fibrillation, 42 Yorkshire farm pigs weighing an average of 34 ± 2 kg were randomized to receive standard CPR (SCPR, n = 12), SCPR + IPC (n = 10), SCPR + IPC + CVT (n = 10), or SCPR + CVT (n = 10). IPC was delivered during the first 3 min of CPR with 4 cycles of 20 s of chest compressions followed by 20-s pauses. CVT consisted of intravenous sodium nitroprusside (2 mg) and adenosine (24 mg) during the first minute of CPR. Epinephrine was given in all groups per standard protocol. A transthoracic echocardiogram was obtained on all survivors 1 and 4 h post-ROSC. The brains were extracted after euthanasia at least 24 h later to assess ischemic injury in 7 regions. Ischemic injury was graded on a 0–4 scale with (0 = no injury to 4 ≥50% neural injury). The sum of the regional scores was reported as cerebral histological score (CHS). 48 h survival was reported.

Results: Post-resuscitation left ventricular ejection (LVEF) fraction improved in SCPR + CVT, SCPR + IPC + CVT and SCPR + IPC groups compared to SCPR (59% ± 9%, 52% ± 14%, 52% ± 14% vs. 35% ± 11%, respectively, $p < 0.05$). Only SCPR + IPC and SCPR + IPC + CVT, but not SCPR + CVT, had lower mean CHS compared to SCPR (5.8 ± 2.6, 2.8 ± 1.8 vs. 10 ± 2.1, respectively, $p < 0.01$). The 48-h survival among SCPR + IPC, SCPR + CVT, SCPR + IPC + CVT and SCPR was 6/10, 3/10, 5/10 and 1/12, respectively (Cox regression $p < 0.01$).

Conclusions: IPC and CVT during standard CPR improved post-resuscitation LVEF but only IPC was independently neuroprotective and improved 48-h survival after 15 min of untreated cardiac arrest in pigs.

1. Introduction

An estimated 350,000 patients suffer from out-of-hospital cardiac arrest (OHCA) each year in the United States (151). Even with the best clinically documented methods of cardiopulmonary resuscitation (CPR), more than 85–90% of OHCA patients die or have

severe neurological deficits (152). Cerebral and cardiac dysfunction following successful resuscitation from cardiac arrest is the major cause of death and long-term morbidity (153,154).

We recently have shown that after 15 min of untreated cardiac arrest due to ventricular fibrillation, ischemic postconditioning (IPC) at the initiation of standard CPR, can improve neurological intact survival (74). IPC with four, 20 s pauses during the first 3 min of CPR have been shown to be synergistic with sodium nitroprusside "enhanced" CPR (SNPeCPR) which utilizes active compression and decompression CPR with an inspiratory impedance threshold device and abdominal binding (76).

In this investigation we try to build upon our previous published studies and evaluate the effects of cardioprotective vasodilator therapy (CVT) alone and in combination with IPC in a model of standard CPR (SCPR). We sought to provide evidence of reduced global reperfusion injury after prolonged ischemia with histological and biomarker based endpoints in addition to the clinical endpoints.

We hypothesized that by using a simple CPR strategy designed to control the initial reintroduction of blood flow during Basic Life Support (BLS), we could protect vital organs from injury and substantially improve outcomes after 15 min of untreated ventricular fibrillation. Further, we hypothesized that the addition of cardioprotective vasodilatory agents would act synergistically with IPC.

2. Materials and methods

All studies were performed on Yorkshire farm pigs weighing an average of 34 ± 2 kg. A certified and licensed veterinarian provided a blinded neurologic assessment at 24 and 48 h. The Institutional Animal Care Committee of the Minneapolis Medical Research Foundation approved the protocol (number 11-05, approved on 5/10/2011) (155).

2.1. Preparatory phase

The anesthesia, surgical preparation, data monitoring, and recording procedures used in this study have been described previously in detail and the study protocol was used unaltered from Segal et al (74). After endotracheal intubation, inhaled isoflurane at a dose of 0.8–1.2% was used for anesthesia up until ventricular fibrillation (VF) induction.

Anesthesia was restarted after return of spontaneous circulation (ROSC). The animal's bladder temperature was maintained at 37.5 ± 0.5 ˚C with a warming blanket (Bair Hugger, Augustine Medical, Eden Prairie, MN). Central aortic and right atrial pressures were recorded continuously with micromanometer-tipped catheters (Mikro-Tip Transducer, Millar Instruments, Houston, TX). The left internal carotid artery was surgically exposed and an ultrasound flow probe (Transonic 420 series multichannel, Transonic Systems, Ithaca, NY) placed to quantify blood flow (mL/min). Compression force, rate and depth, were continuously recorded throughout all experiments and controlled during CPR to assure all groups received identical CPR quality.

2.2. Experimental protocol

After the surgical preparation was complete, oxygen saturation on room air was >95%, and $ETCO_2$ was stable between 35 and 42 mmHg for 5 min, VF was induced by delivering direct intracardiac current. Standard chest compression cardiopulmonary resuscitation was performed with a pneumatically driven automatic piston device (Pneumatic Compression Controller, Ambu International, Glostrup, Denmark) as previously described (126). During SCPR, we delivered uninterrupted chest compressions at a rate of 100 compressions/min, with a 50% duty cycle and a compression depth of 25% of the anteroposterior chest diameter. Asynchronous positive-pressure ventilations were delivered with room air (FIO_2 of 0.21) with a manual resuscitator bag. The tidal volume was maintained at ~10 mL/kg and the respiratory rate was 10 breaths/min. The investigators were blinded to hemodynamics during CPR. If ROSC was not achieved, defibrillation was delivered every 2 min thereafter during CPR. Resuscitation efforts were continued until ROSC was achieved or for a total of 15 min.

2.3. Protocol

We tested two interventions in this study, independently, and in combination during SCPR. These interventions included, (a) IPC, that was delivered with four cycles of 20-s pauses for the first 3 min of the resuscitation effort (74) and (b) administration of cardioprotective vasodilator therapy (CVT). CVT consisted of sodium nitroprusside (SNP) and adenosine. SNP was given as a 2 mg bolus at minute 1 and a second 1 mg bolus at minute 3 of CPR (76). Adenosine was given as a single 24 mg bolus after the first SNP

bolus (after preliminary studies demonstrated superiority of this dose in improving post resuscitation left ventricular (LV) dysfunction (76). Epinephrine was administered in all groups in a 0.5 mg (~15 g/kg) bolus at minute 4 of CPR, 60 s before first defibrillation.

Following 15 min of untreated VF, 42 pigs were randomized prospectively using a computer-generated program into four groups:

I. SCPR group as controls: received only SCPR and epinephrine (12 animals).

II. IPC group: received four 20-s pauses during the first 3 min of SCPR.

III. CVT group: received SNP and adenosine as described above while performing SCPR.

IV. IPC + CVT group: received both four 20-s pauses of CPR (IPC) and CVT, as described above. Groups II–IV had 10 animals each.

2.4. Post-ROSC care

The protocol for post resuscitation care has been described in detail by Segal et al (74). Supplemental oxygen was added only if arterial saturation was lower than 90%. Animals were observed under general anesthesia with isoflurane until hemodynamically stable. Hemodynamic stability was defined as a mean aortic pressure >55 mmHg without pharmacologic support for 10 min as well as normalization of $ETCO_2$ and acidosis.

Animals that were hypotensive post-ROSC received increments of 0.1 mg intravenous epinephrine every 5 min until mean arterial pressure rose above 50 mmHg. If pH was lower than 7.20, 50–100 mEq of $NaHCO_3$ were given intravenously. All groups received post-resuscitation therapeutic hypothermia as recommended by the American Heart Association for comatose patients resuscitated from VF to simulate best practice and optimize the chances of the control group for neurological recovery (156). Target temperature was set at 34 ˚C and was maintained at that level with the use of a cutaneous cooling device (Arctic Sun, Medivance Inc., Louisville, CO). Central temperature was measured at the bladder of the animals. Total hypothermic time was 12 h (156).

Survivors were given intramuscular injections of nonsteroidal analgesics (84). Animals were returned to their runs and were observed every 2 h for the first 6 h for signs of distress or accelerated deterioration of their function If animals met predetermined criteria of an adverse outcome such as status epilepticus, severe cardio-respiratory distress or deep coma after 24 h based on the judgment of a veterinarian, blinded to the intervention,

they were euthanized per protocol.

2.5. Neurologic assessment

24 and 48 h after ROSC, a certified veterinarian, blinded to the intervention, assessed the pigs' neurologic function based on a modified cerebral performance category (CPC) scoring system for pigs. That has been described in detail by our group.5 The following scoring system was used: 1 = normal; 2 = slightly disabled; 3 = severely disabled but conscious; 4 = vegetative state; and 5 was given to animals that died in the lab due to unachievable ROSC or died in the run following ROSC.

2.6. Echocardiographic evaluation of the left ventricle

A transthoracic echocardiogram was obtained on all survivors 1 and 4 h post-ROSC. Parasternal long and short axis views were obtained at each time point. Ejection fraction (EF) was assessed by visual estimation from two independent clinical echocardiographers blinded to the treatments and if there was more than 10% difference between the two readings, the EF was calculated by using Simpson's method of volumetric analysis (128).

2.7. Neurohistology

Animals that were found dead without direct observation were excluded from histological analysis due to unknown time of death. At 48 h (or if the veterinarian decided that the animals needed to be sacrificed after the 24 h evaluation) animals underwent sedation, were intubated, and anesthetized as described previously. Surgical exposure and cannulation of both common carotid arteries were performed under full anesthesia protocol.

An IV bolus of heparin (3000 units) was administered. Animals were then sacrificed by an injection of 10 mEq potassium chloride via direct cardiac stick. Immediately upon sacrifice, normal saline was infused through the carotid sheaths via a peristaltic pump (Cole Parmer, Vernon Hills, IL). The head was removed from the body, and 10% buffered formalin pumped through the carotid sheaths. The brain was allowed to fix for 1–3 h prior to removal and then the whole brain was removed and placed in 10% buffered formalin solution.

After fixation with formalin, brains were stained with hematoxylin and eosin (H&E). Assessment was modeled after a previously published method (120).

Each pig brain was sectioned coronally into 5 slices (excluding brainstem and cerebellum) with the first cut at the level of the mammillary bodies for consistency. Within the 5 brain slices, sections sampled included: (1) frontal cortex, including watershed zones and underlying structures at the level of the head of the caudate; (2) deep gray nuclei (caudate putamen and globus pallidus), temporal cortex and hippocampus at the level of the mammillary bodies; (3) hippocampus and temporal cortex at the level of thalamus (including thalamus), (4) midbrain, (5) pons, (6) medulla and (7) cerebellum (1 cm from the midline including watershed zones). When regions were represented at more than one level, scoring was based on the section where damage was more extensive and notations made if significant differences are noted at two levels (i.e. head and body of caudate or anterior and posterior hippocampus). Light microscopic evaluations were made using an Olympus microscope. A board certified human neuropathologist blinded to the group treatments performed the histological evaluations at the core laboratories of the University of Minnesota.

We used a semi-quantitative scale for ischemic injury. Seven specific brain regions were graded on a 0–4 scale (0: no injury, 1–4, mild to severe). A score of 0 indicated that less than 1% ischemic neurons were seen in the region, 1 (minimal) was used if fewer than 10% of neurons appeared ischemic, 2 (mild) was used if 10–25% of neurons were ischemic, 3 (moderate) was used for 26–50% ischemia, and 4 (marked) for more than 50% ischemic neurons. The sum of categorical scores for regional prevalence throughout the brain describes the overall prevalence of ischemic neurons and is described here as total Cerebral Histological Score (CHS)

2.8. Cardiac biomarkers

A 3-ml sample of arterial blood was obtained from all survivors 4 h after ROSC. Cardiac-specific troponin-I and creatinine phosphokinase-MB (CK-MB) were quantified via a two-site sandwich assay (Stratus CS Acute Care, Siemens, Tarrytown, NY). Personnel performing the analyses were blinded to treatment.

2.9. Statistical analysis

Values are expressed as mean ± SD. The primary end point was the incidence of major adverse outcomes at 48 h. The adverse outcomes were defined by the protocol as

death or euthanasia after the veterinarian's recommendation due to status epilepticus, severe cardiorespiratory distress with evidence of agonal breathing with cyanosis or pulmonary edema and coma at 24 h with the inability to respond to painful stimuli. Secondary endpoints were (a) 4-h left ventricular ejection fraction (LVEF) and (b) the cerebral histological score. A single-factor ANOVA was used to determine statistical significance of differences in means of continuous variables between groups. Pairwise comparison of subgroups was performed with the Student–Newman–Keuls test. Significance was set at a value of $p < 0.05$. A Kaplan Maier curve was plotted to show freedom from major adverse outcomes (death or euthanasia after 24 h due to coma, refractory seizures and cardio-respiratory distress) in the intervention and control groups. Cox regression analysis was used to assess the effect of IPC, and CVT on major adverse outcomes up to 48 h. The computer generated randomization process allocated the numbers of the animals in each group prospectively.

3. Results

All animals were included in the analysis. No adverse event occurs during the experiment. There were no significant baseline differences between treatment groups (Tables 3-1.1 and 3-1.2).

3.1. Hemodynamics and arterial blood gasses

During the first 3 min of CPR all animals had similar hemodynamics except, as expected, during the periods where pauses of compression and ventilations were introduced (Table 3-1.1). CVT therapy did not significantly affect coronary perfusion pressures. Arterial blood gases during CPR and at recovery are shown in Table 3-1.2.

3.2. ROSC and 48-h incidence of major adverse outcomes

There were no significant differences in ROSC between groups (Table 3-1.1). IPC treated animals had a significant decrease in the combination of death and pre-specified major adverse outcomes (coma at 24 h, refractory seizures and cardio-respiratory distress leading to euthanasia) during the 48 h of observation. IPC was independently and strongly associated with a decrease in the risk of death and major adverse outcomes [HR = 0.13, 95% CI = 0.035, 0.53, p = 0.004] and there was no observed synergy between IPC and

CVT (Fig. 3-1.1, Table 3-1.3). In the same fashion, IPC ($p = 0.01$) and IPC + CVT ($p = 0.024$) were superior to SCPR. CVT alone was not superior to SCPR.

3.3. Left ventricular function

Echocardiographic evaluation at 1 h revealed that animals receiving CVT, CVT + IPC or IPC had a significantly higher LVEF than those treated with SCPR (57 ± 12%, 54 ± 18%, 56 ± 14% vs. 36 ± 5%, respectively, $p < 0.01$ for all compared to SCPR). These results were sustained up to 4 h after ROSC (59 ± 9%, 52 ± 7%, 52 ± 14% vs. 35 ± 11%, respectively, $p < 0.05$). There was no difference in the LVEF among CVT, IPC-CVT or IPC alone groups, either at 1 or 4 h (Table 3-1.1).

3.4. Cardiac biomarkers

Plasma was obtained at 4 h in all animals. IPC, CVT + IPC and CVT alone resulted in significantly lower CK-MB and Troponin-I levels (all in g/L) at 4 h compared to SCPR controls. The Troponin-I level was 31 ± 34 at 4 h in SCPR as compared to 8.5 ± 7, 7 ± 7, 5 ± 5 for IPC, CVT, IPC + CVT, respectively ($p < 0.05$). CK-MB was measured at 37 ± 24 in SCPR group compared to 13 ± 10, 18 ± 13, 11 ± 9 for IPC, CVT and IPC + CVT groups, respectively ($p < 0.05$). There was no difference the cardiac biomarkers among the intervention groups.

3.5. Neurologic function

Blinded assessment of cerebral performance category (CPC) at 24 h on the live animals showed improvement in the CVT, CVT-IPC and IPC groups compared to SCPR group (2.6 ± 0.9, 2.25 ± 1, 2.75 ± 0.4 vs. 3.5 ± 0.5, respectively, $p < 0.05$ for all). At 48 h, there was only one animal that survived in the SCPR group and had CPC score of 3 (severe deficit). Surviving animals treated with CVT, CVT + IPC, and IPC alone at 48 h had a mean CPC score of 2.3 ± 1.6, 1.8 ± 0.8, 2.2 ± 0.9 with no difference between the groups, but with improvement in all intervention groups at 48 h compared with 24 h.

3.6. Neurohistopathology

The mean time for brain harvest was shorter in the SCPR group since more animals died earlier or had major adverse outcomes requiring euthanasia. The mean harvest time for SCPR was 20 ± 12 h compared to 39.0 ± 12.4, 38.4 ± 13 and 42.0 ± 12.0 h for IPC, CVT + IPC, CVT, respectively. Despite later evaluation of histopathological samples, IPC

and IPC + CVT resulted in significantly lower total cerebral histological score (CHS) compared to SCPR group (5.8 ± 2.6, 2.8 ± 1.8 vs. 10 ± 2.1, respectively, $p < 0.01$) (Fig. 3-1.2). One animal in the IPC group and two animals in the IPC + CVT group showed no ischemic damage at 48 h (≤2 total CHS). CVT alone did not improve CHS (6.6 ± 3.7) compared to SCPR group ($p = 0.1$).

4. Discussion

Results from this investigation demonstrate that cardiac and cerebral function can be preserved after prolonged global ischemic insult of 15 min of untreated ventricular fibrillation cardiac arrest by early application of ischemic post-conditioning and use of cardioprotective vasodilators during CPR. These findings provide, for the first time, strong support for a simple BLS strategy that includes four controlled pauses of compressions during the first 3 min of CPR improves post-resuscitation LV function. This strategy also decreases the levels of cardiac biomarkers of injury at 4 h and leads to a significant decrease of the ischemic histological injury of the brain leading to better neurological outcomes at 24 and 48 h compared to standard CPR.

In this study, ROSC was not a predictor of neurological and cardiac function post-resuscitation. This finding that is consistent with large human studies showing dissociation between ROSC rates and improved survival with good neurological function (19,20).

Our data suggest that IPC is the most important factor leading to a significant improvement in cardiac function and survival with good neurological function. IPC decreased that risk of death and major adverse events by almost 80% compared to SCPR. It was also associated with significant improvement in cerebral histological injury; a combination of improved survival and cerebral preservation is currently considered the ultimate outcome for any intervention evaluated during cardiac arrest.

Our study is also in concordance with the published study by Wang et al. that showed that IPC was effective in protecting the brains of rats from a global 10-min ischemic insult that were not in cardiac arrest (13). This new observation should cause reassessment of the notion that cerebral recovery is not feasible after 10–12 min of cardiac arrest (136). In a recent series of papers, Allen et al. have shown that controlled reperfusion of the brain with

the use of bypass and a special reperfusion solution that mitigates reperfusion injury can result in the absence of ischemic changes in the brain even after 30 min of isolated global cerebral ischemia (157,158). Based on similar principles, we have provided a simple method of IPC (with four 20-s pauses in compressions and ventilation during the first 3 min of CPR) that could be easily applied in the clinical setting and translated to patients receiving CPR for treatment of cardiac arrest.

We used IPC during CPR because of the described mechanisms of protection from reperfusion injury which currently is thought to be modulation of the opening of mitochondrial permeability transition pores (mPTP) and K_{ATP} channels (16,140,143,14,159,160). Our results support the contention that the previously described protective mechanisms can also be observed during the global ischemia and reperfusion of cardiac arrest, decreasing vital organ injury as documented, in this study, with the histological evaluation of the brain and functional and biomarker assessment of the surviving animals.

CVT with sodium nitroprusside and adenosine did not improve neurological outcomes but led to an independent improvement in post-resuscitation cardiac function and lower levels of biomarkers of cardiac injury. This is consistent with published evidence supporting the protective properties of these two medications after cardiac ischemia (30). CVT therapy did not show synergy with IPC in clinical outcomes but there was an improvement in cerebral histological score and the IPC + CVT group provided animals with the lowest scores recorded in histopathology analysis of the brain, effectively showing no ischemic changes in any of the regions examined.

By contrast, CVT alone has been shown to be very effective in improving outcomes when it is applied on CPR strategies that provide superior hemodynamics such as active compression decompression CPR and the impedance threshold device in combination with abdominal binding (7). In this setting, the CPR method can increase vital organ perfusion pressures and blood flow and lead to improved outcomes compared to SCPR (76). It became evident in our present study, which used SCPR, that CVT does not offer any significant survival advantage, although it did improve cardiac function after ROSC and there were no detectable side effects.

Our study has limitations. We did not perform a dosing study so we cannot comment on the optimal IPC strategy, or the optimal CVT dosing. Dosing studies are difficult in a large animal laboratory due to the large number of animals needed. We did not evaluate mechanism in this study. We did, nevertheless, establish that our observed improved clinical outcomes were associated with evidence of cardiac and cerebral protection from ischemic injury in this global ischemic model of cardiac arrest. The histological assessment was performed in different times between the standard and the intervention groups. It is well established that histological evidence of ischemia becomes more pronounced after 24 h and progressively gets worse up to 48-h (161). The observed differences in total cerebral histological score therefore are very likely to be an underestimation since there was significantly more time in the intervention groups to develop the observed ischemic changes.

5. Conclusion

A simple IPC strategy applied at the initiation of CPR significantly improved clinical, histopathological and biomarker-related cardiocerebral outcomes in a porcine model of cardiac arrest of prolonged, untreated ventricular fibrillation. CVT offered a significant and independent improvement in post-resuscitation cardiac function and decreased levels of cardiac biomarkers at 4 h post-ROSC but it did not show any independent or synergistic effects with IPC effects on neurological and survival outcomes.

Conflict of interest statement

Demetris Yannopoulos MD, is the Medical Director of the Minnesota Resuscitation Consortium, a state wide initiative to improve survival in the state of MN from cardiac arrest. This initiative is sponsored by the Medtronic Foundation and is part of the Heart Rescue Program. There are no conflicts related to this investigation. Dr. Aufderheide has grants from NIHBI for the Resuscitation Outcomes Consortium, the ResQTrial, and the Immediate Trial; a grant from NINDS for the Neurological Emergency Treatment Trials (NETT) Network; he completed a paid consultancy on an acute myocardial infarction study with Medtronic in November, 2011; he volunteers on the Board of Directors for Take Heart

America, President, Citizen CPR Foundation and is a volunteer for the National American Heart Association on the Basic Life Support Subcommittee and Research Working Group. He has no conflicts related to this investigation. The rest of the authors have no conflicts related to the study.

Ethical approval

The study was approved by the Institutional Animal Care Committee of the Minneapolis Medical Research Foundation of Hennepin County Medical Center, and all animals received treatment in compliance with the National Research Council's 1996 Guide for the Care and Use of Laboratory Animals.

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CPR method	Measurements	Baseline	2 min CPR	4 min CPR	1 h ROSC	4 h ROSC	Number of shocks	Post-ROSC EPI	ROSC
SCPR	SBP	104 ± 18	44 ± 14	68 ± 12	107 ± 4	101 ± 10	6 ± 1	2 ± 1.2	9/12
	DBP	73 ± 13	14 ± 7	29 ± 7	67 ± 15	67 ± 9			
	RA	4 ± 2	3 ± 3	5 ± 3	1 ± 2	4 ± 2			
	CPP	69 ± 15	11 ± 4	24 ± 6	66 ± 11	63 ± 4			
	CBF	201 ± 47	26 ± 5	21 ± 6	113 ± 26	137 ± 16			
	LVEF	N/A	N/A	N/A	36 ± 5	35 ± 11			
SCPR + IPC	SBP	94 ± 30	73 ± 31	75 ± 15	95 ± 19	83 ± 8	2 ± 1*	0.8 ± 0.2*	10/10
	DBP	66 ± 22	22 ± 14	35 ± 11	68 ± 12	59 ± 9			
	RA	5 ± 2	2 ± 1	6 ± 3	9 ± 3	8 ± 1			
	CPP	61 ± 27	20 ± 15*	29 ± 15	58 ± 14	51 ± 11			
	CBF	182 ± 83	39 ± 3*	56 ± 27*	214 ± 49*	227 ± 86*			
	LVEF	N/A	N/A	N/A	56 ± 14*	52 ± 14*			
SCPR + CVT	SBP	91 ± 13	41 ± 15	72 ± 9	92 ± 9	73 ± 10	4 ± 3	1 ± 0.2*	10/10
	DBP	65 ± 8	15 ± 6	35 ± 9	69 ± 9	55 ± 8			
	RA	5 ± 2	3 ± 3	8 ± 3	8 ± 3	7 ± 2			
	CPP	60 ± 8	12 ± 8	27 ± 13	61 ± 9	48 ± 17			
	CBF	178 ± 43	48 ± 19*	45 ± 19*	264 ± 72*	167 ± 66			
	LVEF	N/A	N/A	N/A	57 ± 12*	59 ± 9*			
SCPR + IPC + CVT	SBP	98 ± 11	57 ± 21	72 ± 15	87 ± 13	88 ± 9	6 ± 2	1.4 ± 0.5	9/10
	DBP	69 ± 7	18 ± 12	32 ± 12	64 ± 9	58 ± 7			
	RA	5 ± 2	6 ± 3	4 ± 4	4 ± 1	6 ± 2			
	CPP	64 ± 9	12 ± 5	28 ± 18	60 ± 11	52 ± 12			
	CBF	168 ± 35	67 ± 30*	49 ± 17*	263 ± 66*	218 ± 53*			
	LVEF	N/A	N/A	N/A	54 ± 18*	52 ± 7*			

Table 3-1.1. The hemodynamics, ROSC and survival during CPR, ROSC, post-ROSC and 48 h. Values are shown as mean ± SD. CPR was performed with either SCPR, SCPR + IPC, SCPR + CVT or SCPR + IPC + CVT. All pressures are in mm Hg and all flows in mL/min. CBF, carotid blood flow; CPP, coronary perfusion pressure; DBP, diastolic blood pressure; EPI, epinephrine; RA, right atrial pressure; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction (%). The dose of EPI is in milligrams. * Means statistically significant difference compared to SCPR group with a p value of <0.05.

CPR method	Measurement	Baseline	5-min CPR	30 min ROSC	4 h ROSC
SCPR	pH	7.44 ± 0.03	7.28 ± 0.2	7.26 ± 0.08	7.39 ± 0.06
	PCO_2	42 ± 2	49 ± 13	47 ± 10	41 ± 3
	PO_2	96 ± 14	100 ± 34	98 ± 28	80 ± 25
	HCO_3^-	28 ± 2	22 ± 3	20 ± 2	24 ± 3
	$ETCO_2$	40 ± 2	43 ± 14	39 ± 5	41 ± 5
SCPR + IPC	pH	7.45 ± 0.04	7.26 ± 0.08	7.32 ± 0.03	7.43 ± 0.06
	PCO_2	40 ± 5	47 ± 4	38 ± 4*	41 ± 2
	PO_2	90 ± 20	89 ± 14	83 ± 26	75 ± 20
	HCO_3^-	28 ± 1	21 ± 3	21 ± 2	27 ± 3
	$ETCO_2$	39 ± 3	45 ± 7	35 ± 3	35 ± 2
SCPR + CVT	pH	7.45 ± 0.03	7.29 ± 0.06	7.34 ± 0.03*	7.40 ± 0.07
	PCO_2	43 ± 2	51 ± 5	43 ± 6	42 ± 3
	PO_2	83 ± 19	97 ± 25	86 ± 26	76 ± 18
	HCO_3^-	29 ± 1	25 ± 5	23 ± 2	26 ± 4
	$ETCO_2$	40 ± 1	44 ± 8	37 ± 3	35 ± 4
SCPR + IPC + CVT	pH	7.46 ± 0.04	7.25 ± 0.08	7.35 ± 0.04*	7.46 ± 0.06
	PCO_2	40 ± 3	49 ± 9	40 ± 2*	39 ± 5
	PO_2	83 ± 21	104 ± 21	78 ± 19	89 ± 16
	HCO_3^-	29 ± 2	22 ± 4	22 ± 2	28 ± 2
	$ETCO_2$	38 ± 3	44 ± 5	34 ± 4	34 ± 5

Table 3-1.2. Arterial blood gasses during cardiopulmonary resuscitation and after return of spontaneous circulation. Mean ± SD. Arterial blood gas measurements at baseline and after ROSC. Partial pressures in torr. SaO_2, percent oxygen saturation; HCO_3, bicarbonate; $ETCO_2$, end-tidal CO_2. * Means statistically significant difference compared to SCPR group with a p value of <0.05.

Treatment	HR ± SE	*p*-Value	CI
SCPR	Ref	Ref	Ref
IPC	0.2 ± 0.1	0.01	0.05–0.7
CVT	0.55 ± 0.3	0.26	0.19–1.5
IPC + CVT	0.3 ± 0.2	0.02	0.08–0.8

Table 3-1.3. Effects of IPC, and CVT on major adverse outcomes up to 48 h (Cox regression analysis). SCPR is the reference. CI, confidence interval; HR, hazard ratio; and Ref, reference.

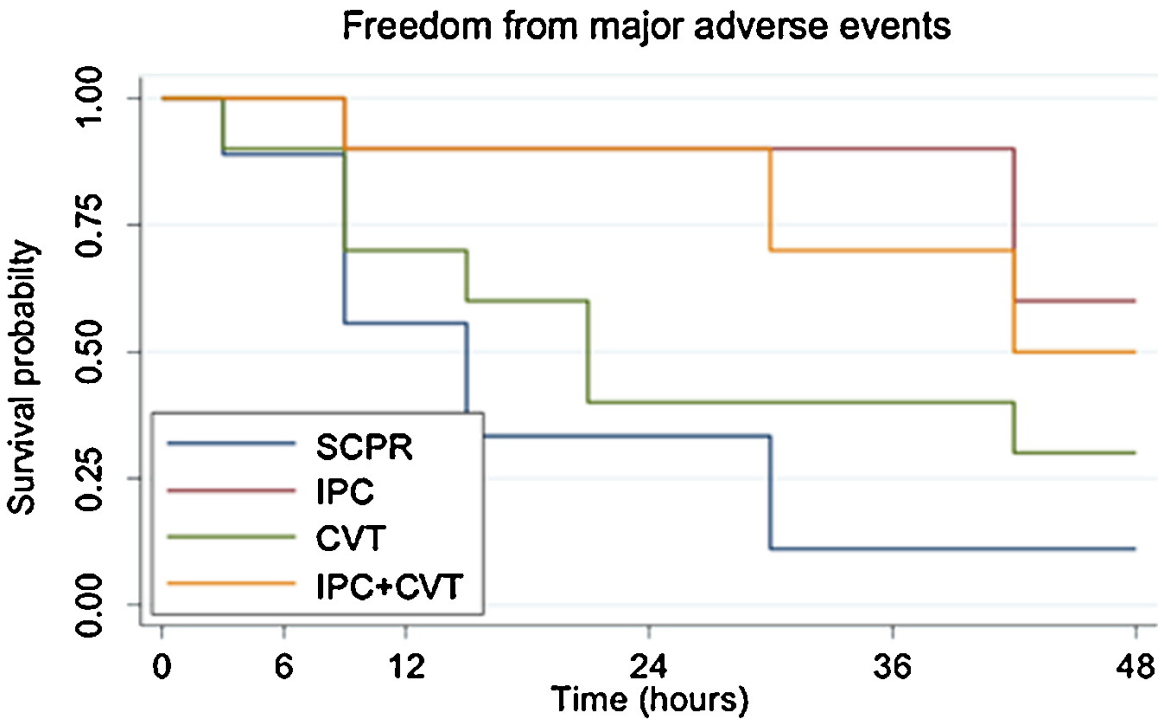

Figure 3-1.1. Kaplan–Meier curve of all 4 groups including only animals that achieved ROSC. The data demonstrate a significantly lower incidence of major adverse outcomes at 48 h post-ROSC with IPC and IPC + CVT interventions compared with SCPR ($p = 0.0097$). (Major adverse outcomes are defined as death or coma, refractory seizures and cardio-respiratory distress leading to euthanasia.) SCPR, standard CPR; IPC, ischemic post-conditioning; and CVT, cardioprotective vasodilatory therapy.

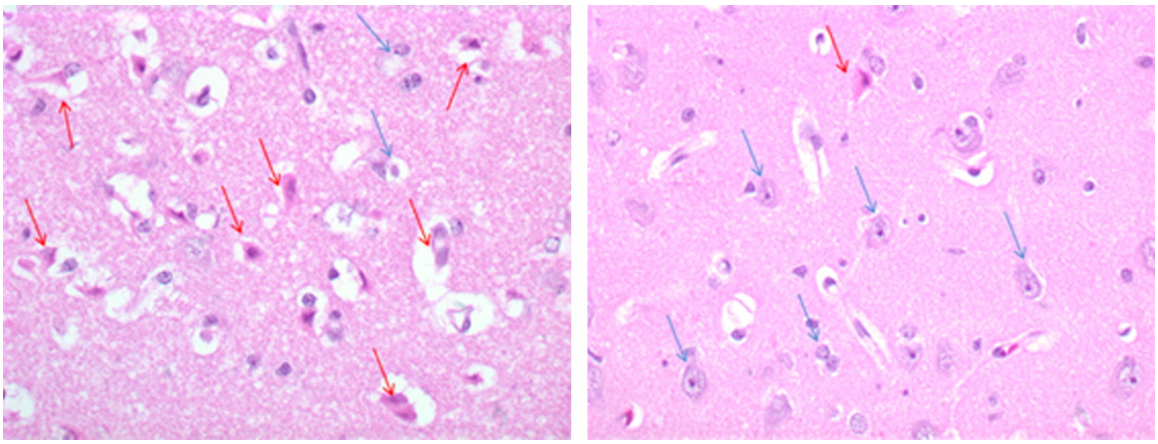

Figure 3-1.2. Left panel: severe ischemic brain injury (SCPR), right panel: minimal ischemic brain injury (IPC) representative samples of severe versus minimal histologic ischemic brain injury. Purple condensed nuclei are a marker of ischemic insult (red arrow). Healthy neurons are shown with blue arrow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

CHAPTER 3
PART 2

Bundled postconditioning therapies improve hemodynamics and neurologic recovery after 17 min of untreated cardiac arrest

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Synoposis

Objective: Ischemic postconditioning (stutter CPR) and sevoflurane have been shown to mitigate the effects of reperfusion injury in cardiac tissue after 15 min of ventricular fibrillation (VF) cardiac arrest. Poloxamer 188 (P188) has also proven beneficial to neuronal and cardiac tissue during reperfusion injury in human and animal models. We hypothesized that the use of stutter CPR, sevoflurane, and P188 combined with standard advanced life support would improve post-resuscitation cardiac and neurologic function after prolonged VF arrest.

Methods: Following 17 min of untreated VF, 20 pigs were randomized to Control treatment with active compression/decompression (ACD) CPR and impedance threshold device (ITD) (n = 8) or Bundle therapy with stutter ACD CPR + ITD + sevoflurane + P188 (n = 12). Epinephrine and post-resuscitation hypothermia were given in both groups per standard protocol. Animals that achieved return of spontaneous circulation (ROSC) were evaluated with echocardiography, biomarkers, and a blinded neurologic assessment with a cerebral performance category score.

Results: Bundle therapy improved hemodynamics during resuscitation, reduced need for epinephrine and repeated defibrillation, reduced biomarkers of cardiac injury and end organ dysfunction, and increased left ventricular ejection fraction compared to Controls. Bundle therapy also improved rates of ROSC (100% vs. 50%), freedom from major adverse events (50% vs. 0% at 48 h), and neurologic function (42% with mild or no neurologic deficit and 17% achieving normal function at 48 h). Conclusions: Bundle therapy with a combination of stutter ACD CPR, ITD, sevoflurane, and P188 improved cardiac and neurologic function after 17 min of untreated cardiac arrest in pigs.

1. Introduction

Over 350,000 people are affected by sudden cardiac death in the United States each year (162) while only 8–10% experience neurologically intact survival (19,20,22). Clinical studies have demonstrated severely limited neurologic outcomes when the cardiac arrest is longer than10 min (119). Likewise, animal studies show that prolonged ventricular fibrillation (VF), lasting 12–13 min, prohibits neurologically intact survival with standard

therapy (120–122).

Further study of global ischemia has revealed two types of injury with distinct mechanisms. The first is related to ischemic damage incurred during the period of arrest. The second is injury induced by tissue reperfusion (10,163,164). In contrast to ischemic injury which occurs prior to the arrival of emergency medical services, reperfusion injury, the injury caused by re-establishing blood flow, is amenable to medical management as it occurs when therapy begins.

Recent studies in animal models of cardiac arrest provide promising evidence for improved cardiac function and neurologic recovery when therapies targeting reperfusion injury are used. Ischemic postconditioning, which utilizes structured pauses in CPR, was shown to improve left ventricular ejection fraction (LVEF)and neurologically intact survival in a porcine model of cardiac arrest including 15 min of untreated VF (74). Sevoflurane has been shown to reduce release of cardiac biomarkers, reduce apoptosis, and improve LVEF; however, no difference in neurologic function was reported (112,165). Poloxamer 188 (P188) is a nonionic copolymer thought to adhere to gaps in the cell membrane, thereby blocking pores caused by severe cellular stress (166).

While each of these treatments is likely to provide protection against cardiac and neuronal reperfusion injury induced by cardiac arrest, we hypothesized that combining these therapies would provide a synergistic benefit even in severe injury. We therefore combined these therapies in a porcine model of cardiac arrest including 17 min of untreated VF. The tissue damage induced by this duration of cardiac arrest has generally been considered irreversible. We assessed hemodynamic parameters, markers of cardiac injury and function, neurologic recovery, and freedom from serious adverse events.

2. Materials and methods

All studies were performed with approval from the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation and the National Research Council's Guidelines for the Care and Use of Laboratory Animals. Yorkshire farm pigs weighing 38.6 ± 0.4 kg were used.

2.1. Preparatory phase

The anesthesia, data monitoring and recording, and surgical preparation have been described in detail previously (74). The femoral artery and right external jugular vein were cannulated percutaneously with eight French sheaths to provide access for continuous hemodynamic monitoring and repeated blood tests.

2.2. Hemodynamic monitoring

Surface electrocardiographic tracings were recorded continuously. Central aortic and right atrial pressures were recorded continuously with micromanometer-tipped catheters (Millar Instruments) placed at the proximal descending thoracic aorta via the femoral artery and right atrium via the right external jugular vein, respectively. Coronary perfusion pressure (CPP) was calculated as the difference between the diastolic aortic pressure and right atrial pressure during the decompression phase of CPR. End tidal CO_2 ($ETCO_2$), blood oxygen saturation, tidal volume, and minute ventilation were continuously monitored with a respiratory monitor (CO_2SMO Plus, Novametrix Medical Systems). All data were recorded with a digital recording system (BIOPAC MP150, BIOPAC Systems Inc.).

2.3. Experimental protocol

The animals were allowed to stabilize with oxygen saturation >95% and ETCO2between 35 and 42 mmHg for 5 min. Isoflurane anesthesia was stopped 5 min prior to VF induction with direct intracardiac current delivered via a temporary pacing wire (St. Jude Medical). Following 17 min of untreated VF, 20 pigs were randomized to Control or Bundle treatment resulting in 8 pigs in the Control group and 12 pigs in the Bundle group.

3. Experimental groups (Fig. 3-2.1):

Control therapy: Active compression/decompression (ACD) CPR was delivered using a pneumatically driven automated piston (Pneumatic Compression Controller, Ambu International) with chest compressions occurring at a rate of 100 per minute. Compression depth was maintained at 25% of the anteroposterior chest diameter. Compression force, rate, and depth were continuously recorded and controlled. An impedance threshold device (ITD; ResQPOD, Advanced Circulatory Systems Inc.) was used in addition to ACD CPR. Asynchronous positive-pressure ventilation was delivered during CPR with room air. A

tidal volume of 10 mL kg^{-1} was delivered at 10 breaths min^{-1}. Isoflurane was not restarted until return of spontaneous circulation (ROSC) was achieved. At minute 3 of CPR all animals received 0.015 mg kg^{-1} of epinephrine. Up to three 275 J biphasic shocks were delivered at minute 4. If ROSC was not achieved, CPR continued with epinephrine administered every 3 min. Defibrillation was attempted every 2 min until ROSC was achieved or a total of 15 min of CPR was complete. If ventricular arrhythmias developed after ROSC was achieved, defibrillation was attempted and amiodarone 40 mg IV was given. Sodium bicarbonate (50 meq) was given to all animals at 5 min of resuscitation.

Bundle therapy: After 17 min of untreated VF, resuscitation began with ischemic postconditioning including stutter ACD CPR + ITD composed of 20 s of compressions followed by a 20 s pause. Sevoflurane was delivered during each pause at an end-tidal concentration of 2.0 vol.% with three positive pressure ventilations at a rate of 10 breaths min^{-1}. No ventilations were delivered while compressions were performed. Once three cycles of stutter ACD CPR + ITD were complete, continuous chest compressions were performed similar to the Control group. Continuous ventilation with 10 breaths min^{-1} was initiated 20 s later. P188 (250 mg kg^{-1}) was delivered at minute 2 of resuscitation. Epinephrine (0.015 mg kg^{-1}) was given at minute 3 of the resuscitation. Defibrillation was attempted at minute 4 as discussed above. If ROSC was achieved, P188 (460 mg kg^{-1}) was infused over 4 h.

3.1. Post-ROSC care

The details of post-ROSC care have been described in detail elsewhere (74). Both groups received post-resuscitation therapeutic hypothermia to simulate best practice and optimize neurologic recovery. All animals received 1 L chilled saline (8–10˚C) immediately post-ROSC followed by surface cooling with ethanol soaked towels. Target temperature (34˚C) was maintained for 4 h with the use of external cooling (Arctic Sun, Medivance Inc.). Animals were rewarmed at 0.5˚C/h to 36˚C. In the event of an adverse event meeting predetermined criteria including status epilepticus, severe cardiopulmonary distress with evidence of agonal breathing, cyanosis, pulmonary edema, or deep coma at 24 h with the inability to respond to painful stimuli based on the judgment of the veterinarian blinded to the intervention, animals were euthanized.

3.2. Echocardiographic evaluation

A transthoracic echocardiogram was obtained at baseline and again at 15 min, 1, 4, 24, and 48 h post-ROSC. Parasternal long and short axis views were obtained and analyzed by clinical echocardiographers blinded to the intervention as described previously (74).

3.3. Cardiac biomarkers, liver function tests, and renal function assessment

Arterial blood was collected at baseline and from all survivors 4 h post-ROSC. Cardiac specific troponin I (cTnI) and creatinine phosphokinase MB (CK-MB) were quantified via a two-site sandwich assay (Stratus CS Acute Care, Siemens). Aspartate aminotransferase, alanine aminotransferase, total bilirubin, and creatinine were measured with standard human assays. Personnel performing the analyses were blinded to the treatment.

3.4. Neurological assessment

Neurologic function was assessed using a swine-specific cerebral performance category (CPC) at 24 and 48 h post-ROSC by a certified veterinarian blinded to the treatment. Clinical assessment of behaviors, reflexes, and coordination are assessed as previously described (74) using the following scoring system: 1 = normal, 2 = slightly disabled, 3 = moderately disabled but not comatose, 4 = coma, 5 = dead.

3.5. Statistical analysis

Values are expressed as means ± standard error of the mean. The primary end points were the incidence of major adverse out-comes at 48 h and the CPC at 24 and 48 h. Secondary endpoints included hemodynamics, need for epinephrine and defibrillations, LVEF post-ROSC, and biomarkers. Baseline data, hemodynamic parameters, epinephrine and defibrillation requirements, LVEF, biomarkers, and mean CPC scores were compared using two-tailed unpaired t-tests. Kaplan–Meier survival curves were analyzed via Mantel–Cox test. A p-value of <0.05 was considered statistically significant.

4. Results

All results were obtained after 17 min of untreated VF. There were no significant differences in baseline hemodynamic parameters between treatment groups (Fig. 3-2.2).

4.1. Resuscitation hemodynamics

Systolic and diastolic blood pressures were greater with Bundle therapy vs. Controls at minutes 2–4 of CPR (Fig. 3-2.2A). This difference did not persist once ROSC was achieved. CPP was significantly lower with Bundle therapy compared to Control therapy early in the resuscitation while the increasing aortic pressures observed with Bundle therapy translated into higher CPP at minute 3 of the resuscitation (Fig. 3-2.2B). The duration of CPR was significantly reduced in the Bundle therapy group (5.0 ± 0.04 min vs.11.3 ± 1.5 min, p = 0.0001).

4.2. Hemodynamic and rhythm support

The Bundle therapy group required less epinephrine (0.57 ± 0.08 mg vs. 1.34 ± 0.23 mg, p = 0.002; Fig. 3-2.3A) and fewer shocks to achieve and maintain sinus rhythm compared to the Control group (4.75 ± 0.6 vs. 7.5 ± 1.0, p = 0.019; Fig. 3-2.3B). The use of amiodarone was not significantly different between the groups (13.33 ± 5.7 mg vs. 30 ± 6.6 mg, p = 0.07).

4.3. Left ventricular ejection fraction

LVEF was higher with Bundle therapy compared to Control therapy at 15 min post-ROSC (63 ± 3.6% vs. 45 ± 10%, p = 0.009; Fig. 3-2.4). Bundle animals maintained a significantly higher LVEF at 4-h post-ROSC compared to Control animals (57.3 ± 2.1% vs. 42 ± 8.8%). LVEF remained normal in Bundle therapy animals that survived 48 h. No Control animals survived to 48 h for comparison. Baseline LVEF was similar between the groups (58.3 ± 0.7% vs. 58.8 ± 0.8%, p = 0.89).

4.4. Biomarkers

CK-MB, cTnI, creatinine, and the transaminases were significantly reduced with Bundle therapy compared to Controls at 4 h post-ROSC (Table 3-2.1). All baseline values were similar between groups and within normal limits.

4.5. ROSC and freedom from major adverse events

ROSC was achieved in 100% of Bundle animals and 50% of Controls (Fig. 3-2.5A and Fig. 3-2.6). 75% of Bundle animals remained free of major adverse events at 24 h compared to 25% of Controls. Overall, 50% of all Bundle therapy animals remained free of major adverse events at 48 h, whereas no Control animals remained free of adverse events at 48 h (p = 0.005).

4.6. Neurologic function at 24 and 48 h

Bundle therapy animals demonstrated significantly better neurologic function at 24 h (Fig. 3-2.5B; mean CPC score: 3.4 ± 0.4 vs.4.8 ± 0.2, p = 0.015) and 48 h (mean CPC score: 3.4 ± 0.5 vs. 5.0 ± 0.0, p = 0.019). Both Control animals had severe disability (CPC score of 4) at 24 h while neither of them remained free of major adverse events at 48 h. 33% of Bundle animals that remained free of major adverse events at 24 h had good neurologic function (CPC 1-2) while 33% had moderate disability. 67% of Bundle animals free of major adverse events at 24 h remained free from major adverse events at 48 h with 50% of those demonstrating improved neurologic function between 24 and 48 h. Of all Bundle animals, 42% had good neurologic function (CPC 1-2) at 48 h post-ROSC.

5. Discussion

This study shows, for the first time, successful resuscitation resulting in good cardiac and neurologic function after 17 min of untreated VF cardiac arrest using a combination of CPR and medications that could be incorporated into basic and advanced life support protocols. Bundle therapy improved hemodynamics during resuscitation, reduced the need for epinephrine and defibrillation, preserved cardiac ejection fraction, reduced biomarkers of cardiac ischemia, improved freedom from major adverse events, and improved the cerebral performance post-resuscitation. Some animals achieved complete recovery to normal neurologic function.

The most important findings of this study are the large improvement in freedom from major adverse events and the extent of neurologic recovery observed despite 17 min of untreated VF. ROSC rates were significantly lower in Control animals despite 15 min of high quality CPR and continued defibrillation attempts. Of the 12 Bundle animals, 42% had good neurologic function with only slight disability while 17% recovered to have normal neurologic function within 48 h of resuscitation. Thus, 17 min of ischemia was not sufficient to preclude recovery to normal neurologic function. Instead, it was the reperfusion process that determined the potential for neurologic recovery. This implicates reperfusion injury as an important factor in damage from prolonged cardiac arrest.

We have previously shown normal cardiac and neurologic function after 15 min of

untreated VF arrest using stutter CPR (74). Allen et al. (157,158) also showed almost complete neurologic recovery after 30 min of untreated isolated cerebral ischemia using a pig model with controlled reperfusion using high pressure and a reperfusate that was hyperosmolar, alkalotic, depleted of calcium, and enriched with magnesium (167). However, systemic ischemia may significantly alter the pathology.

Normalization of neurologic function with Bundle therapy may be related to hemodynamic improvements. Intracranial pressure was not measured in this study; however, prior studies using this model observed intracranial pressures between 20 and 30 mmHg (168). Given the elevated aortic pressures achieved in the animals receiving Bundle therapy, cerebral perfusion pressures in excess of 70 mmHg may have been achieved which may contribute to the improved neurologic recovery observed.

Stutter CPR has previously been shown to improve hemodynamics after 15 min of VF arrest (74). However, the mean systolic blood pressure of 150 mmHg in the current study exceeds that previously seen suggesting synergism with sevoflurane and P188. Bundle therapy resulted in intra-resuscitation systolic pressures that exceeded baseline and post-ROSC pressures. This has not been observed with other resuscitation protocols. The etiology is unknown. The improvement in hemodynamics reduced the need for epinephrine. Epinephrine can reduce microcirculatory blood flow including cerebral perfusion (169) while also increasing myocardial oxygen demand (170) and ventricular ectopy (171). Therefore, reducing epinephrine use may provide additional benefits to post-resuscitation cardiac and neurologic function.

Diastolic blood pressure also increased to baseline levels with Bundle therapy which likely improved cardiac function by increasing CPP. This may contribute to the normal post-ROSC LVEF and lower biomarkers of cardiac ischemia observed in Bundle animals.

Improved hemodynamics are unlikely to account for all benefits observed with Bundle therapy, as ischemic postconditioning, sevoflurane, and P188 are known to have effects on cellular processes including membrane stability and mitochondrial function. Ischemic postconditioning is believed to protect mitochondria from the effects of prolonged ischemia by inhibiting the opening of the mitochondrial permeability transition pore which is known to trigger apoptosis in the setting of ischemia and reperfusion (138–

141). Improved myocardial function has been observed when ischemic postconditioning was used in the setting of myocardial infarction and cardiac arrest (74,75,143). Improved neurologic function has also been observed with use of ischemic postconditioning in the setting of ischemic stroke (142) and more recently in global ischemia such as that experienced during cardiac arrest (13,74,75).

Sevoflurane has been shown to confer endothelial protection by reducing leukocyte activation while also affecting vascular tone (172). Though the specific mechanisms remain unclear, sevoflurane has previously been shown to improve myocardial function, prevent apoptosis, and reduce cytokine production post-cardiac arrest (112).

The mechanism of P188 in cardiac arrest is entirely untested. P188 is hypothesized to fill ischemia-induced pores in the plasmamembrane (166). This may prevent unregulated exchange of ions between the extracellular and intracellular compartments thereby preventing cellular injury and apoptosis (173,174). P188 has been shown to protect neurons (175) and skeletal muscle (176,177) against ischemia/reperfusion injury. It also preserves blood-brain barrier integrity during traumatic brain injury thereby reducing brain edema and neuronal apoptosis (178). P188 has shown mixed results during myocardial infarction in humans. Schaer et al. (179) showed reduced infarct size, increased LV function, and reduced in-hospital re-infarction rates when P188 was infused immediately after thrombolytic therapy. However, a large follow-up study showed no benefit of P188 following acute myocardial infarction (180). This discrepancy is not fully understood but it may relate to proximity of the infusion to reperfusion.

Studies of acute myocardial infarction noted that P188 worsened renal function acutely in up to 4% of patients (180). It has been hypothesized that creatinine, a marker of glomerular filtration rate, is artificially increased by P188 as they compete for excretion in the kidneys. However, renal function in this study, indicated by creatinine levels, was significantly improved in the pigs receiving Bundle therapy including P188. This improvement may be due to improved hemodynamics leading to improved renal perfusion.

This study has important limitations. The role of individual components of Bundle therapy cannot be determined. The Bundle strategy was chosen based on the observation that treatment of complex pathology (e.g. heart failure, acute myocardial infarction, and

stroke) often requires multiple agents. Agents with known or probable benefits were chosen for inclusion in Bundle therapy with a preference for those that may be integrated into standard advanced life support. However, further study will now be necessary to more fully understand the contribution and mechanism of each individual constituent. In addition, the dose-response relationships are unknown for the individual components. The specific regimen of stutter CPR was chosen based on prior published experience (74). The doses of sevoflurane and P188 were extrapolated from animal studies and human trials. Further dose-response trials will be necessary to understand the pharmacokinetics and maximal effect achievable in the setting of cardiac arrest. Similarly, further study is necessary to understand the role of these agents in cardiac arrests of very long or short duration, as the benefit of one or more of these therapies may depend on the duration of ischemia. Lastly, these studies are inherently limited by use of a porcine model. The benefits for humans cannot be certain until human trials are conducted.

6. Conclusion

Bundle therapy, combining stutter ACD CPR, an ITD, sevoflurane, and P188, significantly improved neurologic and cardiac function in a porcine model including 17 min of untreated ventricular fibrillation cardiac arrest. Compared to Control animals, animals receiving Bundle therapy demonstrated significant improvement in hemodynamics during CPR, reduction in biomarkers of cardiac ischemia, normalization of ejection fraction, and improved 48 h survival with favorable neurologic function.

Conflict of interest statement

J.M. Metzger is on the scientific advisory board of and holds shares in Phrixus Pharmaceuticals Inc., a company developing novel therapeutics for heart failure. None of the other authors have any financial or personal relationships with people or organizations that could have inappropriately influenced this work.

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Ethical approval

The study was approved by the Institutional Animal Care Committee of the Minneapolis Medical Research Foundation of Hennepin County Medical Center (protocol #12-11), and all animals received treatment in compliance with the National Research Council's 1996 Guide for the Care and Use of Laboratory Animals.

	Control	Bundle
CK-MB	59.2 ± 13.6	28.2 ± 4.2*
TpnI	27.7 ± 7.7	12.0 ± 2.7*
Tbili	0.1 ± 0.0	0.3 ± 0.1
ALT	61.8 ± 2.0	40.6 ± 4.9*
AST	258.3 ± 67.3	124.6 ± 24.0*
Creatinine	1.9 ± 0.1	1.4 ± 0.1*

Table 3-2.1 Serum biomarkers assessed 4 h post-ROSC. Values are shown as mean ± SEM. Asterisks indicate statistical significance ($p < 0.05$). CK-MB = creatine kinase MB; TpnI = troponin I; Tbili = total bilirubin; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

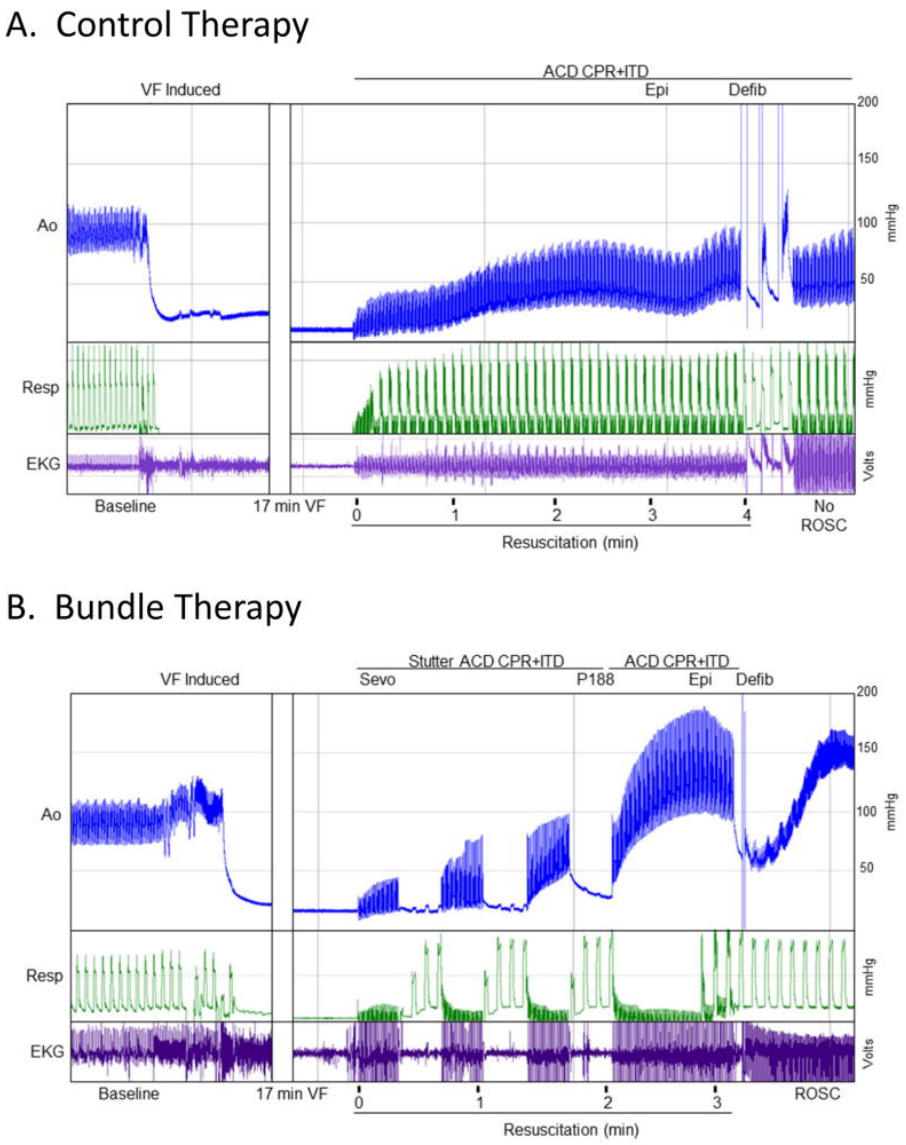

Figure. 3-2.1. Bundle therapy and Control protocols with associated representative hemodynamic tracings. Therapy was initiated after 17 min of untreated ventricular fibrillation(VF). (A) Control therapy included active compression decompression CPR, application of an impedance threshold device (ACD CPR + ITD), epinephrine (Epi), and defibrillation (Defib). Return of spontaneous circulation (ROSC) was not achieved after the initial defibrillation in this representative study leading to continuation of the resuscitation. (B)The Bundle therapy protocol included sevoflurane (Sevo), poloxamer 188 (P188), epinephrine (Epi), stutter ACD CPR + ITD, and defibrillation (Defib). ROSC is achieved after a single defibrillation in this representative study.

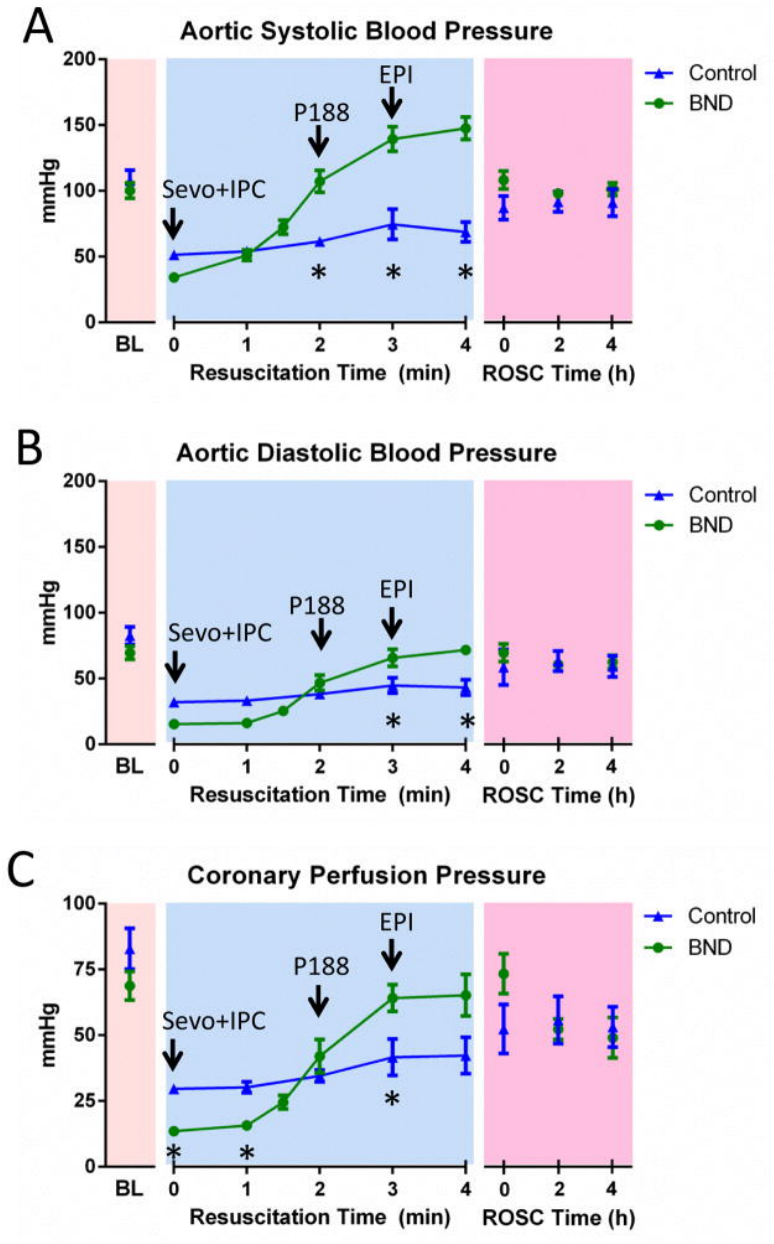

Fig. 3-2.2. Hemodynamic monitoring during resuscitation. Bundle (BND; n = 12) and Control (n = 8) therapy are compared. (A) Aortic systolic blood pressure measured at baseline (BL), throughout the resuscitation, and after return of spontaneous circulation (ROSC) was achieved. (B) Aortic diastolic blood pressure measured during all phases of resuscitation. (C) Coronary perfusion pressure is shown as measured during all phases of resuscitation. Error bars represent SEM. Asterisk (*) indicates statistical significance ($p < 0.05$).

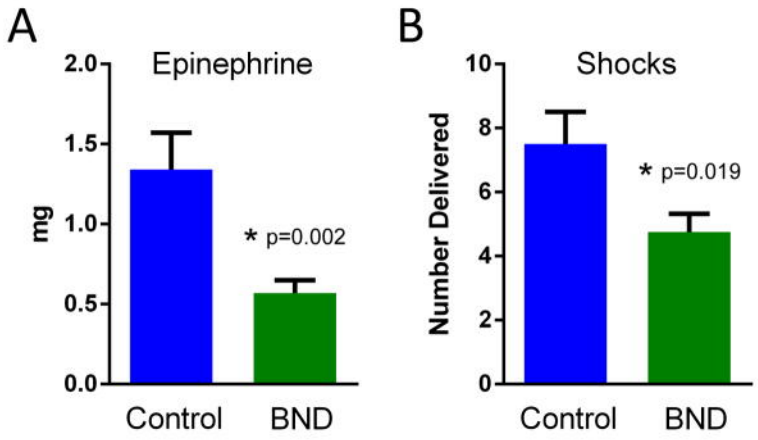

Figure 3-2.3. Epinephrine and defibrillation requirements during resuscitation. Animals receiving Bundle (BND; n = 12) and Control (n = 8) therapy are shown. (A) The mean of the total epinephrine (mg) required during resuscitation. (B) The mean number of shocks delivered during resuscitation. Error bars indicate SEM. p values are as indicated.

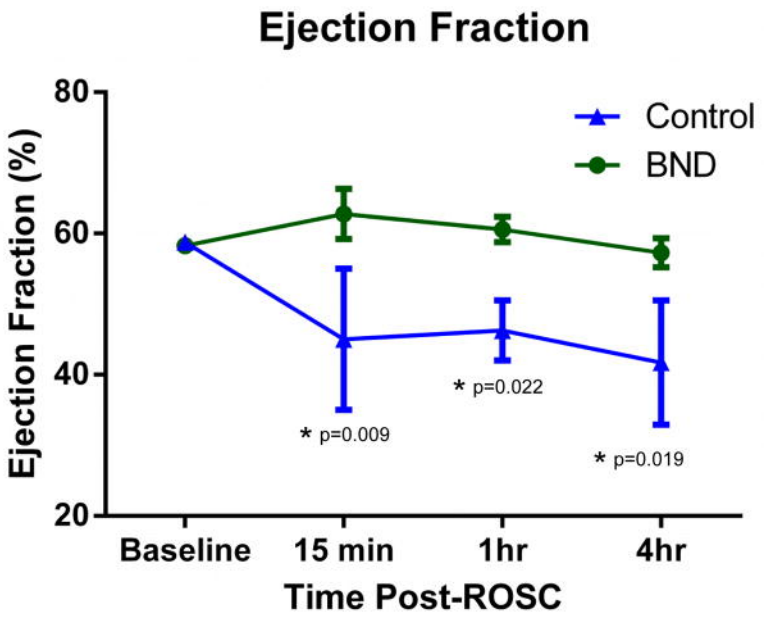

Figure 3-2.4. Serial measurement of the left ventricular ejection fraction (LVEF) for animals receiving Bundle versus Control therapy as determined by echocardiography. All animals that remained free of major adverse events were assessed with echocardiography at the time points noted (BND n = 12 and Control n = 4). Mean LVEF was compared at each time point with asterisks noting statistically significant differences with p-values as indicated.

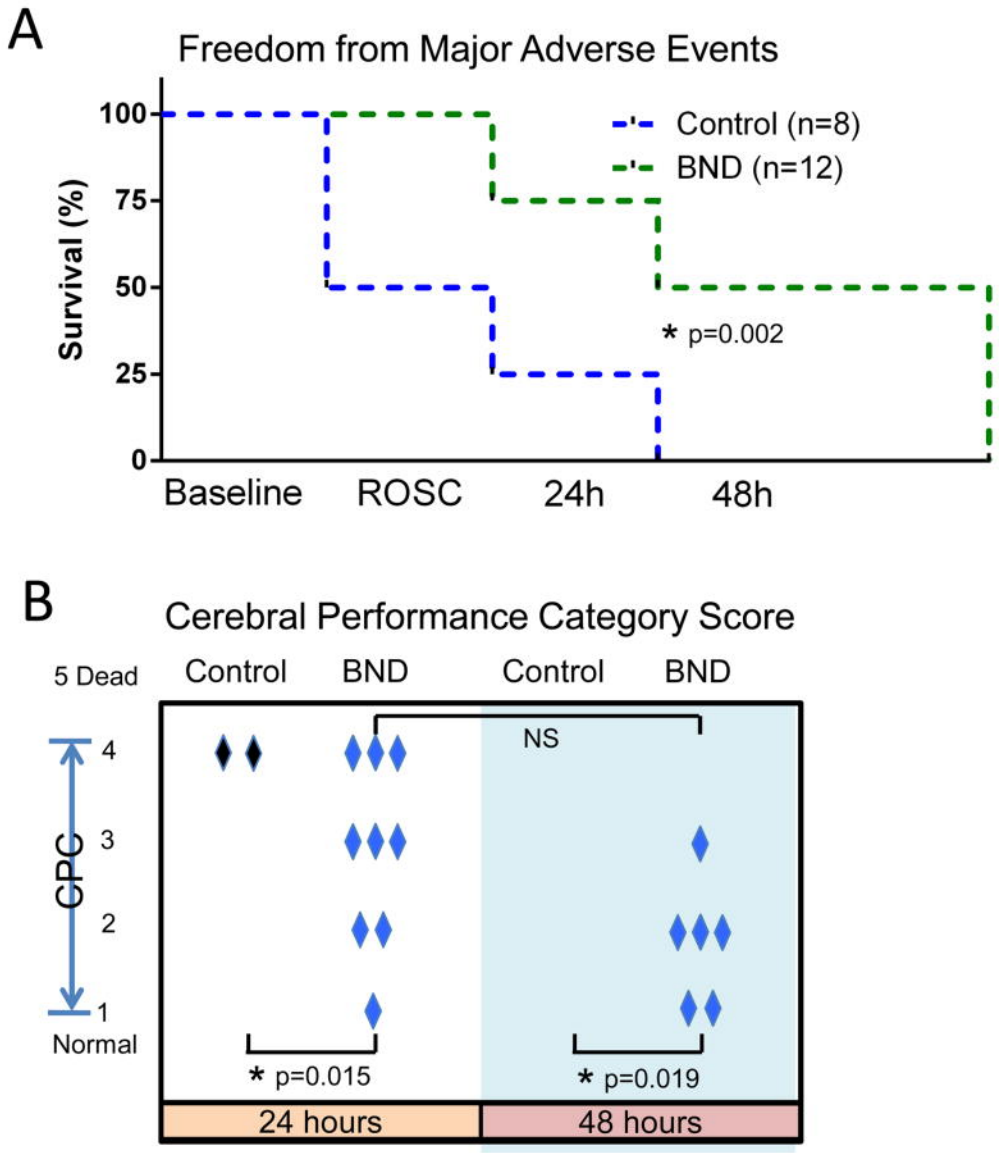

Figure 3-2.5. Clinical outcomes. (A) Kaplan–Meier curve demonstrating freedom from major adverse events during the survival study including baseline, ROSC, and 24and 48 h. Animals receiving Bundle (BND; n = 12) and Control (n = 8) therapy are compared. (B) Cerebral performance category score for each animal that remained free from major adverse events at 24 or 48 h as indicated (1 = normal, 2 = mild deficit, 3 = moderate deficit but conscious, 4 = coma). Mean CPC scores are compared with p-values as indicated. NS indicates that statistical significance was not reached.

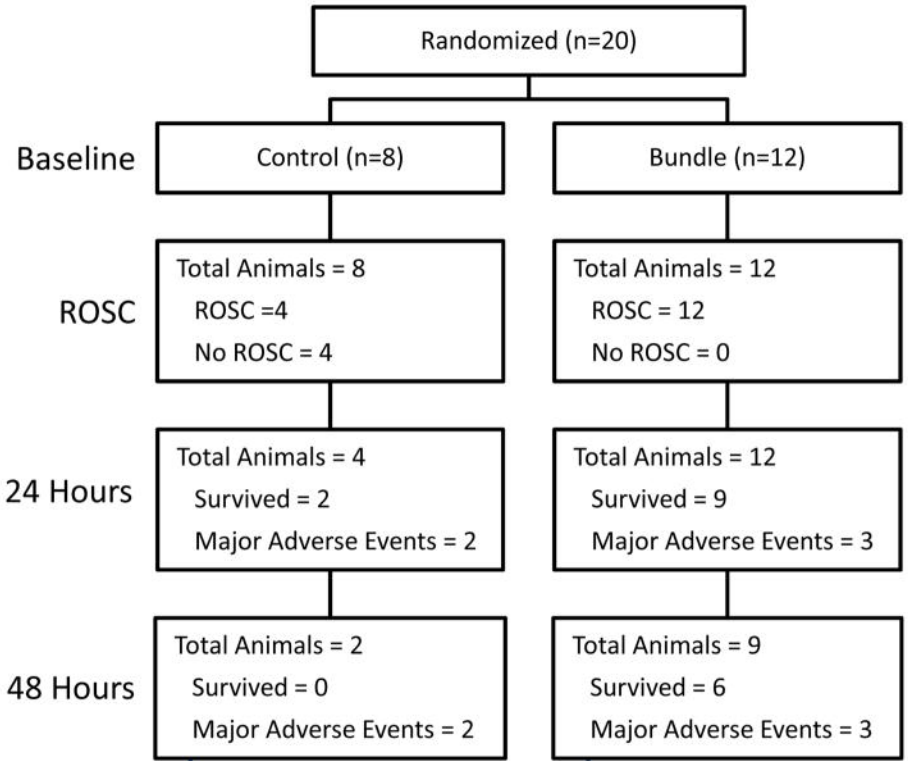

Figure 3-2.6. Survival at all stages of the study. Flow chart describing the survival of animals at the time of randomization, ROSC, 24 and 48 h.

CHAPTER 4

Part 1

Early Effects of Prolonged Cardiac Arrest and Ischemic Postconditioning during Cardiopulmonary Resuscitation on Cardiac and Brain Mitochondrial Function in Pigs

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Synopsis

Background: Out-of-hospital cardiac arrest (CA) is a prevalent medical crisis resulting in severe injury to the heart and brain and an overall survival of less than 10 percent. Mitochondrial dysfunction is predicted to be a key determinant of poor outcomes following prolonged CA. However, the onset and severity of mitochondrial dysfunction during CA and cardiopulmonary resuscitation (CPR) is not fully understood. Ischemic postconditioning (IPC), controlled pauses during the initiation of CPR, has been shown to improve cardiac function and neurologically favorable outcomes after fifteen minutes of CA. We tested the hypothesis that mitochondrial dysfunction develops during prolonged CA and can be rescued with IPC during CPR (IPC-CPR).

Methods: 63 swine were randomized to no ischemia (Naïve), nineteen minutes of ventricular fibrillation (VF) CA without CPR (Untreated VF), or fifteen minutes of CA with 4 minutes of reperfusion with either standard CPR (S-CPR) or IPC-CPR. Mitochondria were isolated from the heart and brain to quantify respiration, rate of ATP synthesis, and calcium retention capacity (CRC). Reactive oxygen species (ROS) production was quantified from fresh frozen heart and brain tissue.

Results: Compared to Naïve, Untreated VF induced cardiac and brain ROS overproduction concurrent with decreased mitochondrial respiratory coupling and CRC, as well as decreased cardiac ATP synthesis. Compared to VF CA, S-CPR attenuated brain ROS overproduction but had no other effect on mitochondrial function in the heart or brain. Compared to VF CA, IPC-CPR improved cardiac mitochondrial respiratory coupling and rate of ATP synthesis, and decreased ROS overproduction in the heart and brain.

Conclusions: Fifteen minutes of VF CA results in diminished mitochondrial respiration, ATP synthesis, CRC, and increased ROS production in the heart and brain. IPC-CPR attenuates cardiac mitochondrial dysfunction caused by prolonged VF CA after only 4 minutes of reperfusion, suggesting that IPC-CPR is an effective intervention to reduce cardiac injury. However, reperfusion with both CPR methods had limited effect on mitochondrial function in the brain, emphasizing an important physiological divergence in post-arrest recovery between those two vital organs.

INTRODUCTION

Out-of-hospital cardiac arrest (CA) afflicts 395,000 people each year in the United States (181). On average, less than 6 percent survive, resulting in more than 360,000 deaths per year and an enormous public health burden (2). In the past five decades, improvements to cardiopulmonary resuscitation (CPR) and systems-based interventions have resulted in only modest improvements to outcomes following prolonged CA. Novel resuscitation strategies are necessary to further improve survival.

One such novel strategy is ischemic postconditioning (IPC), a therapy delivered upon reperfusion after prolonged ischemia to mitigate cellular injury caused by ischemia and reperfusion (IR) (11,182). IPC is accomplished using several brief interruptions in blood flow at the onset of reperfusion. Our laboratory applied IPC during CPR in a porcine model of prolonged whole-body ischemia during ventricular fibrillation (VF) CA. Pauses in chest compressions during the first 2 minutes of CPR improved left ventricular ejection fraction (LVEF) following return of spontaneous circulation (ROSC) and increased neurologically favorable survival after 48 hours (74,75,183). These data demonstrate that the method of initial reperfusion with CPR has an impact on the extent of injury after prolonged cardiac arrest.

Mitochondria are implicated in the pathophysiology of IR injury and also provide a nexus for integrating the protective molecular pathways activated by IPC (184,17,18). Therefore, we sought to investigate the effect that VF, CPR, and IPC have on mitochondrial function in the heart and brain. We hypothesized that mitochondrial function: 1) is significantly depressed after prolonged cardiac arrest, and 2) can be improved with IPC at the initiation of CPR (IPC-CPR). Mitochondrial responses to ischemia, CPR, and IPC were characterized without attempting defibrillations to eliminate confounders such as success and timing of ROSC, refractory VF, number of defibrillations, antiarrhythmics, and vasopressor support. Understanding the early physiological consequences of the key components of cardiac arrest and resuscitation will have significant impact on the direction and refinement of future therapies.

METHODS

All studies were performed with approval from the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation and the University of Minnesota in accordance with the National Research Council's Guidelines for the Care and Use of Laboratory Animals (185). Animal preparation has been described previously (75). See Supplement for full description of methods.

Experimental Protocols

Following surgical preparation and baseline measurements, animals were randomized to one of 4 groups (Naïve, Untreated VF, S-CPR, and IPC-CPR) and further to either heart or brain isolation (Fig 4-1.1). Twelve animals received no ischemia (Naïve, heart isolation [n = 7], brain isolation [n = 5]). In the 3 ischemic groups, VF was induced via a pacing wire positioned in the right ventricle. During VF, ventilation and temperature management were discontinued. Mitochondria were isolated 19 min after initiation of VF in all 3 ischemic groups. To establish the effect of prolonged untreated VF CA, 13 animals were randomized to receive 19 min of VF CA without CPR (Untreated VF, heart [n = 8], brain [n = 5]). To establish the effect of reperfusion by type of CPR on mitochondrial function, 38 animals were randomized to receive 15 min of untreated VF arrest followed by either 4 min of standard CPR (S-CPR, heart [n = 11], brain [n = 8]) or 4 min of IPC-CPR (heart [n = 11], brain [n = 8]). Epinephrine (0.125 μg/kg) was administered after the 3rd minute of CPR. In the IPC-CPR group, IPC was administered via 3 cycles of 20 seconds compressions/ventilations followed by 20 seconds pause in compressions/ventilations during the first 2 minutes of CPR. Both CPR groups received asynchronous ventilations at 10 breaths/min and 10 ml/kg, and 100 chest compressions/min with a target depth of 20% AP diameter and 50% duty cycle. Compressions were performed with an automated, custom-built, CPR piston device (Caztek Engineering, St. Paul, MN). Coronary perfusion pressure (CPP), the gradient between aortic and right atrial blood pressures during chest decompression, was calculated as an approximation of coronary blood flow and a measure of CPR quality. At the end of the 4th minute of CPR, tissue was sampled for mitochondria isolation. No defibrillations were attempted.

At the conclusion of the experimental protocol, mitochondria were isolated at 4°C

via differential centrifugation from the left ventricle of the heart and brain as described previously (186,187). State 3 (S3) and State 4 (S4) respiration, the rate of ATP synthesis, and calcium retention capacity (CRC) were quantified as previously described (186). The respiratory control index (RCI) was calculated as the ratio of S3 to S4 respiration. Production of reactive oxygen species (ROS) was assessed with electron spin resonance (ESR) fresh frozen heart and brain tissue.

Statistics

Values are expressed as mean ± standard error of the mean. ANOVA with Newman-Keuls posthoc test was used for comparisons between treatments. Unpaired two-tailed t-tests were used to compare hemodynamics during CPR between S-CPR and IPC-CPR. The null-hypothesis was rejected for $p < 0.05$.

RESULTS

Baseline hemodynamic parameters did not differ between Naïve, Untreated VF, S-CPR, or IPC-CPR groups (Table 1). As expected, pauses in chest compressions during IPC-CPR resulted in a lower average CPP during each pause compared to S-CPR (Fig 4-1.2a). However, IPC-CPR resulted in a higher CPP during the 4th minute of CPR after administration of epinephrine. Average CPP during CPR did not differ between S-CPR and IPC-CPR (Fig 4-1.2b).

Mitochondrial Function

Mitochondria were isolated from the heart and brain to quantify S3 and S4 respiration, RCI, rate of ATP synthesis, and CRC. ROS production was quantified in fresh frozen heart and brain tissue.

Prolonged Ischemia (Untreated VF)

a. Heart. Compared to Naïve, Untreated VF resulted in a significant decrease in RCI with both complex I and complex II substrates (Fig 4-1.3a,b). The decrease in RCI was predominantly driven by a rise in S4 respiration indicating uncoupling of oxidative phosphorylation (Fig 4-1.3e,f). The rate of ATP synthesis after Untreated VF was less than 50% of Naïve hearts, consistent with the respiration profile described above (Fig 4-1.3g,h).

Untreated VF resulted in decreased CRC with complex II substrates compared to Naïve (Figure 4-1.3j) and caused a significant increase in ROS production compared to Naïve hearts (Fig 4-1.5a).

b. Brain. Compared to Naïve, Untreated VF significantly decreased RCI for complex II substrates (Fig 4-1.4b). Decreased complex II RCI was predominantly driven by a substantial decrease in S3 respiration with no change in S4 respiration (Fig 4-1.4d,f), whereas complex I stimulated S3 and S4 respiration decreased proportionally (Fig 4-1.4c,e). The rate of ATP synthesis was unaffected by Untreated VF compared to Naïve (Fig 4-1.4g,h). Untreated VF resulted in decreased CRC with both complex I and complex II substrates compared to Naïve (Fig 4-1.4i,j). ROS production significantly increased after Untreated VF compared to Naïve brain (Fig 4-1.15b).

Reperfusion with S-CPR

a. Heart. Compared to Untreated VF, 4 minutes of reperfusion with S-CPR lead to a progressive increase of RCI, particularly for complex II substrates, predominantly driven by increased S3 respiration while S4 respiration remained unchanged (Fig 4-1.3a-f). However, RCI remained decreased after reperfusion with S-CPR compared to Naïve due primarily to increased S4 respiration. S-CPR resulted in a non-significant increase in the rate of ATP synthesis compared to Untreated VF (Fig 4-1.3g,h). S-CPR did not affect CRC compared to Untreated VF (Fig 4-1.3i,j). ROS production in the myocardium was similar between S-CPR and Untreated VF and remained elevated compared to Naïve (Fig 4-1.5a).

b. Brain. Four minutes of reperfusion with S-CPR did not alter mitochondrial respiration, ATP synthesis, or CRC compared to Untreated VF (Fig 4-1.4). S-CPR significantly decreased ROS overproduction in the brain compared to Untreated VF to a level that remained elevated compared to Naïve treatment (Fig 4-1.5b).

Reperfusion with IPC-CPR

a. Heart. Compared to Untreated VF and S-CPR, 4 minutes of reperfusion with IPC significantly increased RCI for both complexes I and II to values similar to Naïve (Fig 4-1.3a,b). Increased RCI was a result of significantly decreased S4 respiration combined with

non-significant increased S3 respiration, indicating tighter coupling of oxidative phosphorylation (Fig 4-1.3c-f). The rate of ATP synthesis normalized to the level of Naïve hearts and was significantly increased compared to Untreated VF (Fig 4-1.3g,h). IPC-CPR significantly improved CRC with complex I substrates compared to Untreated VF (Fig 4-1.3i) and decreased ROS production in the myocardium compared to both Untreated VF and S-CPR (Fig 4-1.5a).

b. Brain. Four minutes of reperfusion with IPC-CPR did not alter mitochondrial respiration, ATP synthesis, or CRC compared to Untreated VF (Fig 4-1.4). IPC-CPR significantly decreased ROS production in the brain compared to Untreated VF and S-CPR to a level that remained elevated compared to Naïve treatment (Fig 4-1.5b).

DISCUSSION

To our knowledge, this is the first study investigating the effect of IPC during high-quality CPR on mitochondrial function after prolonged CA in swine. To determine their role in the early pathogenesis and recovery after prolonged CA, mitochondrial responses to whole-body ischemia and to CPR reperfusion with and without IPC were characterized during ongoing CPR to eliminate the confounding impact of defibrillation attempts and independent of success or timing of ROSC. After 4 minutes of reperfusion, IPC-CPR attenuated mitochondrial dysfunction compared to Untreated VF and S-CPR. Untreated VF resulted in dysfunctional mitochondrial respiration, CRC, and ROS production in the heart and brain, as well as decreased cardiac mitochondrial ATP synthesis. S-CPR partially restored cardiac mitochondrial respiration and ATP synthesis, but was insufficient to decrease ROS overproduction. In contrast, IPC-CPR normalized cardiac mitochondrial respiration and ATP synthesis to non-ischemic levels. ROS overproduction in the brain decreased with S-CPR and was further attenuated with IPC-CPR, but brain mitochondrial function was otherwise unaffected by reperfusion with either method of CPR.

RCI quantifies the coupling of oxygen consumption to ATP synthesis during the primary mitochondrial function of oxidative phosphorylation. Untreated VF decreased RCI with both complex I and complex II substrates in cardiac mitochondria, consistent with findings that respiratory dysfunction impedes cardiac recovery after prolonged ischemia

(188,189). Compared to Untreated VF, reperfusion with S-CPR increased cardiac mitochondrial RCI with both complex I and complex II substrates. IPC-CPR further increased cardiac mitochondrial RCI compared to S-CPR, demonstrating a tighter coupling of oxygen consumption to ATP synthesis. The increase in cardiac mitochondrial RCI with IPC-CPR occurred in tandem with an increase in the rate of ATP synthesis. Maintenance of cardiac bioenergetics enhances the probability of successful resuscitation (190–192). Tighter coupling of oxidative phosphorylation with IPC-CPR may be critically important for efficient oxygen utilization during the hypoxic conditions of CA and hypoperfusion with CPR, and may thus minimize myocardial damage and preserve LVEF as previously observed (74,75).

Increased intracellular and mitochondrial calcium concentrations promote open conformation of the mPTP (193). Early, prolonged opening of the mPTP during IR is proposed to be a fundamental determinant of cell death (17,194). CRC quantifies the amount of calcium-mediated stress necessary to induce mPTP opening (195). Compared to Naïve animals, Untreated VF decreased CRC with complex II substrates in mitochondria isolated from the heart. Decreased CRC may be due to ROS overproduction (196,197) or may reflect increased mitochondrial calcium levels in vivo during Untreated VF. Reperfusion with S-CPR did not affect CRC, whereas IPC-CPR increased CRC with complex I substrates compared to Untreated VF. Decreased ROS overproduction or decreased in vivo calcium loading could contribute to improved calcium stress tolerance with IPC-CPR, though the identity of this mechanism remains unknown from these studies.

ESR intensity of oxidized aconitase is a marker of mitochondrial ROS production. Oxidized aconitase is inactive (198), potentiating a decreased flux through the TCA cycle. Untreated VF and S-CPR showed a significantly increased oxidized aconitase signal in the heart compared to Naïve. IPC-CPR dramatically reduced cardiac mitochondrial ROS production compared to Untreated VF and S-CPR. Attenuation of ROS overproduction improves cardiac contractility in isolated hearts (199) and could be a mechanism through which IPC improves LVEF after prolonged cardiac arrest (74,75).

In a previous study of cardiac and cerebral recovery in a porcine model of prolonged cardiac arrest, IPC-CPR was shown to improve post-resuscitation LVEF and decreased

incidence of adverse events compared to S-CPR (74,75). In the present study, cardiac mitochondrial respiration decreased dynamically during Untreated VF and S-CPR compared to Naïve and could be rescued early during resuscitation with IPC-CPR. Taken together, these data suggest a positive association between improved mitochondrial function during CPR and post-ROSC cardiac function. These data indicate that CPR is a therapeutic opportunity to modify the severity of cardiac IR injury in agreement with evidence from myocardial infarction that postconditioning therapies applied at the onset of reperfusion confer protection to the myocardium (200,201). S-CPR alone also trended toward an increase in both RCI and rate of ATP synthesis compared to Untreated VF, corroborating the observation that perfusion with CPR improves probability of ROSC after prolonged cardiac arrest (202). Yeh et al. similarly observed preserved cardiac mitochondrial function and viability after reperfusion with CPR compared to time-controlled untreated CA (203). Thus, despite evidence of IR injury as demonstrated by the attenuation of mitochondrial dysfunction with IPC-CPR, establishing blood flow with CPR after prolonged CA should remain a primary goal of resuscitation.

In contrast to the reperfusion response of cardiac mitochondria, brain mitochondrial function declined during Untreated VF and did not respond within the first 4 minutes of reperfusion with S-CPR or IPC-CPR. S-CPR decreased ROS overproduction in the brain compared to Untreated VF, while IPC-CPR further decreased ROS overproduction compared to S-CPR. Untreated VF decreased RCI with complex II substrates and severely depressed CRC with both complex I and complex II substrates in mitochondria isolated from the brain. The rate of ATP synthesis was not significantly affected by treatment. Reperfusion with S-CPR had no effect on CRC in the heart or the brain compared to Untreated VF. The steep and irreversible decline in CRC may be indicative of increased sensitivity to mPTP opening specific to neuronal injury and calcium overload during IR (204–206).

In previous studies, IPC-CPR improved neurologically favorable survival while decreasing neuronal cell death after prolonged cardiac arrest (74,75). IPC also reduced cell death and infarct size in models of cerebral ischemia (142,207). In the present investigation, IPC-CPR did not alter cerebral mitochondrial function compared to

Untreated VF or S-CPR early during resuscitation. This dissociation of early mitochondrial function and neurologic outcome may indicate that IPC-CPR does not confer increased neurologically favorable recovery via improvements in mitochondrial function during reperfusion with CPR. Alternatively, these findings may indicate that cerebral recovery does not occur as early as 4 minutes after the onset of reperfusion. In a recent study characterizing cerebral recovery from asphyxial arrest in swine, mitochondrial respiration remained depressed even after ROSC and 4 hours of recovery (208). Previous experiments demonstrated favorable neurologic outcomes after prolonged CA that continued to improve 24 hours post-ROSC, emphasizing a delayed period of neurologic recovery that may extend well beyond the duration of this investigation (74,75,183).

Heart and brain mitochondrial responses differed dramatically after reperfusion with CPR. Compared to Untreated VF, S-CPR improved coupling of mitochondrial respiration in the heart but had no effect in the brain. S-CPR decreased ROS overproduction compared to Untreated VF in the brain but not in the heart. IPC-CPR improved cardiac mitochondrial RCI and rate of ATP synthesis compared to Untreated VF and S-CPR, but its effect was absent in brain mitochondria. Thus, reperfusion with both CPR methods incrementally improved cardiac mitochondrial performance but had limited effect on mitochondrial function in the brain. This physiological divergence in acute post-arrest recovery may be critical for targeted treatment of these two vital organs.

CPP is an estimate of coronary blood flow. As expected, IPC-CPR decreased CPP during each 20-second pause compared to S-CPR. After completion of the controlled pauses and administration of epinephrine, IPC-CPR increased CPP during the 4th minute of CPR. A threshold perfusion pressure is hypothesized to be critical for predicting successful resuscitation (209). Thus, increased CPP in the 4^{th} minute may be a potential mechanism for improved cardiac mitochondrial function during IPC-CPR. Sensitivity to exogenous epinephrine may indicate that IPC-CPR protects vascular endothelium and smooth muscle. Coronary reflow and endothelial vasodilator response is attenuated after ischemia and reperfusion (11,210), and this response can be salvaged with IPC (211). During prolonged CA, increased revascularization and systemic vasoconstriction may be vital mechanisms through which IPC-CPR improves resuscitation success.

Several limitations to this study need to be acknowledged. The mechanism by which IPC-CPR increased cardiac mitochondrial function was not determined. IPC-CPR increased perfusion pressure during the 4th minute of CPR. Mitochondrial function may have improved as a direct result of obtaining a critical perfusion pressure threshold. Equivalent CPPs could not be generated in the control group to test the effect of blood flow on mitochondrial function. The high CPP obtained in both CPR groups and the lack of underlying coronary disease represent ideal CPR conditions that may limit the clinical translation of these outcomes. Intermittent compressions during IPC-CPR may contribute to variations in chest compliance and thus improve the mechanics of flow generation. The effect of IPC-CPR on thoracic compliance, endothelial function, and coronary flow will be the subject of future investigations. Additionally, the acute decrease in mitochondrial function described here, and its prevention by IPC-CPR, may be only a portion of the pathogenesis of prolonged cardiac arrest. Reperfusion injury can take hours or days to fully manifest. The current data were obtained only 4 minutes after the onset of reperfusion and prior to ROSC to eliminate confounders such as defibrillation attempts, arrhythmias, or refractory VF. Injury may continue to accrue beyond this time, thus contributing to the low incidences of neurologically favorable survival even in patients who experience a ROSC.

CONCLUSIONS

Prolonged untreated CA induced ROS overproduction concurrent with decreased mitochondrial respiratory coupling and CRC in the heart and the brain. Additionally, cardiac mitochondrial ATP synthesis was depressed. S-CPR improved coupling of cardiac mitochondrial respiration, and partially attenuated ROS overproduction in the brain. After 4 minutes of reperfusion, IPC-CPR improved cardiac mitochondrial function as indicated by increased respiratory coupling and rate of ATP synthesis, as well as decreased ROS overproduction. These results indicate potential mechanisms through which IPC-CPR improves cardiac performance following prolonged cardiac arrest. In contrast, IPC-CPR decreased ROS production but did not otherwise improve mitochondrial function in the brain, emphasizing an important physiological divergence in post-arrest recovery between these two vital organs.

SOURCES OF FUNDING

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CONFLICT OF INTEREST STATEMENT

Conflicts of interest: none. None of the authors declares a conflict of interest in relation to this manuscript submission.

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The authors wish to thank Rachel Stark and Qunli Cheng, MD for their valuable contributions.

	Treatment Group			
Parameter	**Naïve**	**Untreated VF**	**S-CPR**	**IPC-CPR**
MAP (mmHg)	80.7 ± 3.7	87.1 ± 6.3	86.7 ± 3.7	88.7 ± 4.7
RA (mmHg)	6.5 ± 0.6	6.0 ± 0.7	5.9 ± 0.7	7.0 ± 0.7
pH	7.46 ± 0.01	7.47 ± 0.01	7.47 ± 0.01	7.46 ± 0.01
pCO_2 (mmHg)	42.7 ± 0.8	41.0 ± 1.1	40.3 ± 0.7	40.5 ± 0.4
pO_2 (mmHg)	96.0 ± 3.8	87.9 ± 4.3	92.9 ± 3.0	97.9 ± 4.0
Temp (˚C)	37.5 ± 0.4	37.2 ± 0.5	37.8 ± 0.2	37.7 ± 0.2
Weight (kg)	41.8 ± 0.7	42.9 ± 1.1	41.6 ± 0.6	42.5 ± 0.7
Number	12	13	19	19

Table 4-1.1. Baseline parameters. There were no differences in hemodynamic or ABG values between treatment groups prior to ischemia. MAP, mean arterial pressure; RAP, right atrial pressure. Data are shown as mean ± SEM.

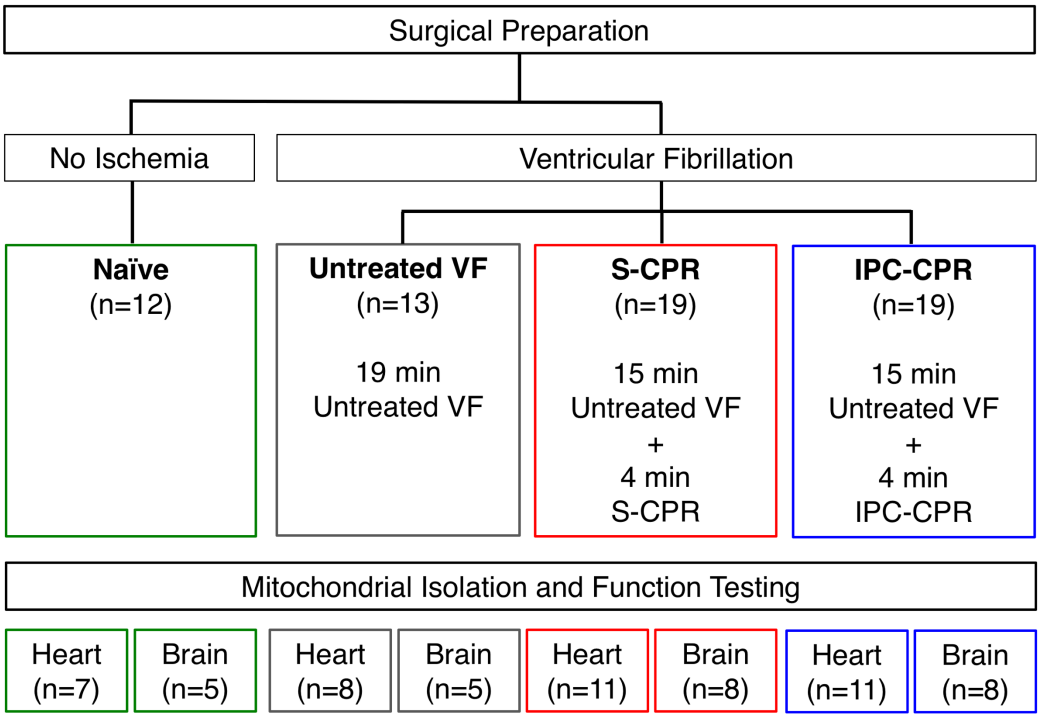

Figure 4-1.1. Experimental Design. Animals were randomized to receive no ischemia (Naïve) or cardiac arrest by ventricular fibrillation (VF), and cardiac arrest groups were further randomized to receive no CPR (Untreated VF), standard cardiopulmonary resuscitation (S-CPR), or ischemic postconditioning at the initiation of CPR (IPC-CPR). After surgical preparation in Naïve or 19 min after induction of VF in ischemic groups, the heart or brain was harvested for isolation of mitochondria via differential centrifugation.

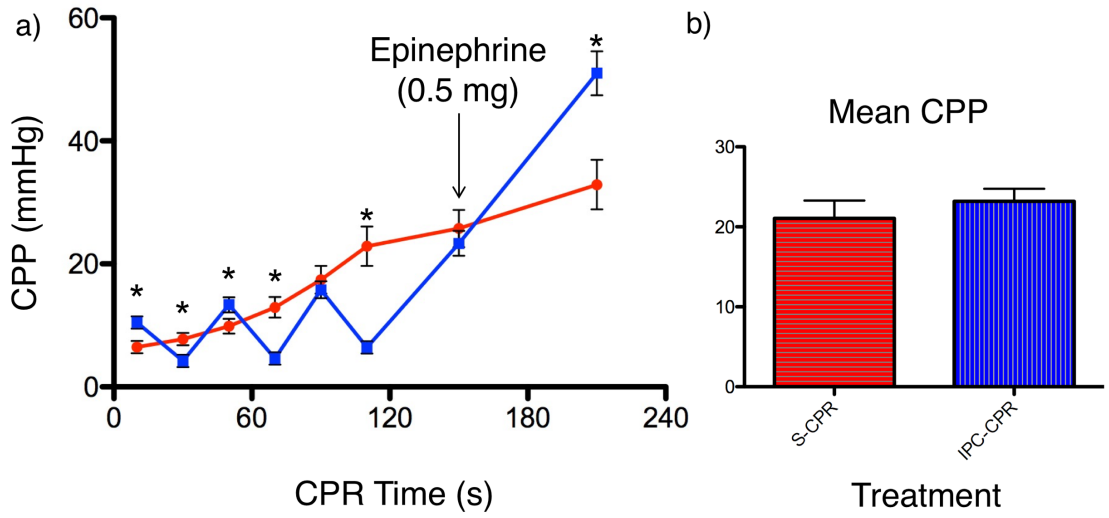

Figure 4-1.2. Coronary perfusion pressure (CPP) during cardiopulmonary resuscitation (CPR) with and without ischemic postconditioning (IPC). IPC was achieved via 3 cycles of 20-seconds compressions followed by 20 seconds pause during the first 2 minutes of CPR. IPC-CPR increased CPP compared to S-CPR following an intravenous bolus of epinephrine (0.125 μg/kg) administered at the start of the 4th minute of CPR (a). The 4-minute CPP average did not differ between groups (b). Data are shown as mean ± SEM. Data from the same time point were compared across treatment with unpaired t-test, * indicates $p < 0.05$.

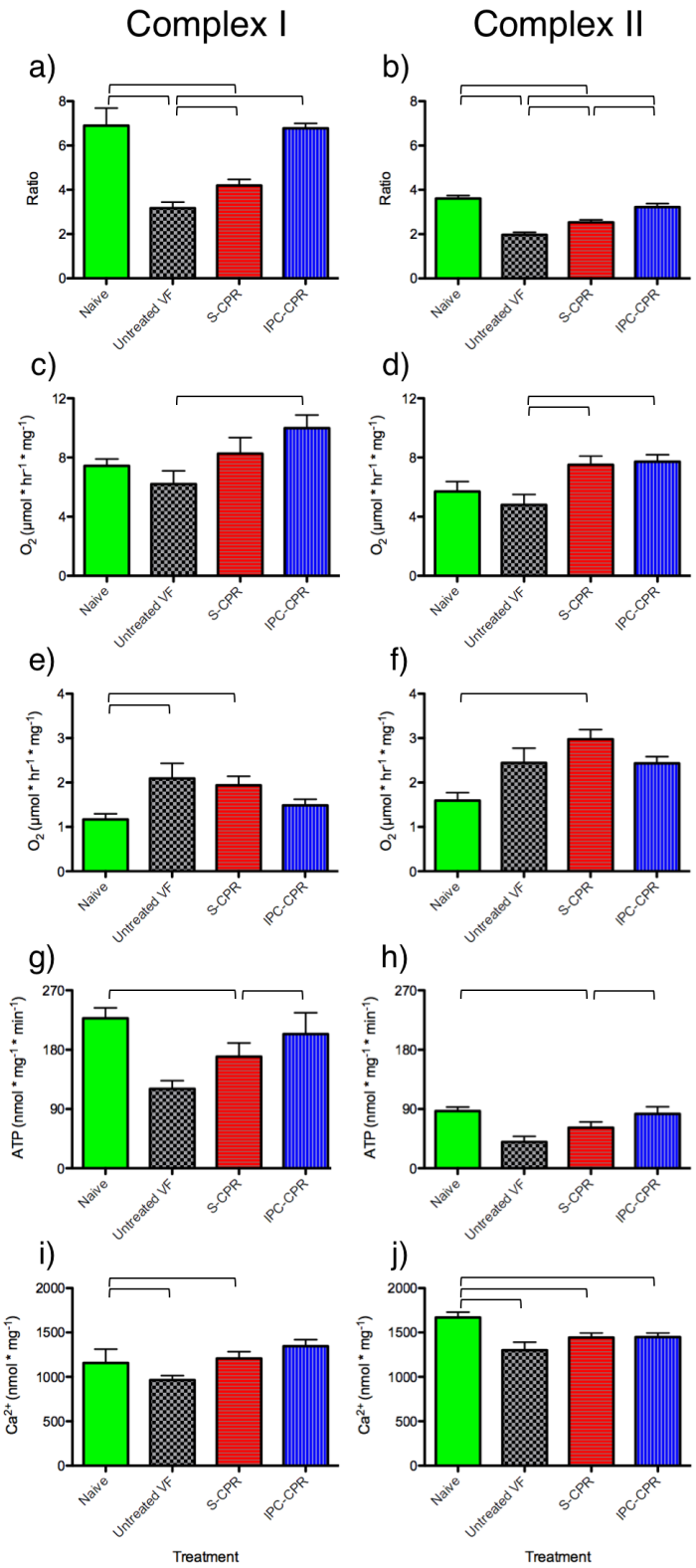

Figure 4-1.3. Cardiac mitochondrial function tests.

Figure 4-1.3. Cardiac mitochondrial function tests. Function tests were assessed with complex I substrates pyruvate and malate (left column) or complex II substrate succinate plus complex I inhibitor rotenone (right column). RCI (a, b) was calculated as the ratio of State 3 (S3; c, d) to State 4 (S4; e, f) respiration. RCI decreased during Untreated VF compared to Naïve primarily due to increased S4 respiration. IPC-CPR increased RCI compared to Untreated VF and S-CPR mediated by increased S3 and decreased S4 respiration. The rate of ATP synthesis decreased during Untreated VF compared to Naïve and was recovered with IPC-CPR (g, h). CRC increased with IPC-CPR compared to Untreated VF in the presence of complex I substrates (i), whereas CRC decreased in all ischemic groups compared to Naïve in the presence of complex II substrates (j). Data are shown as mean ± SEM brackets indicate $p < 0.05$ between groups.

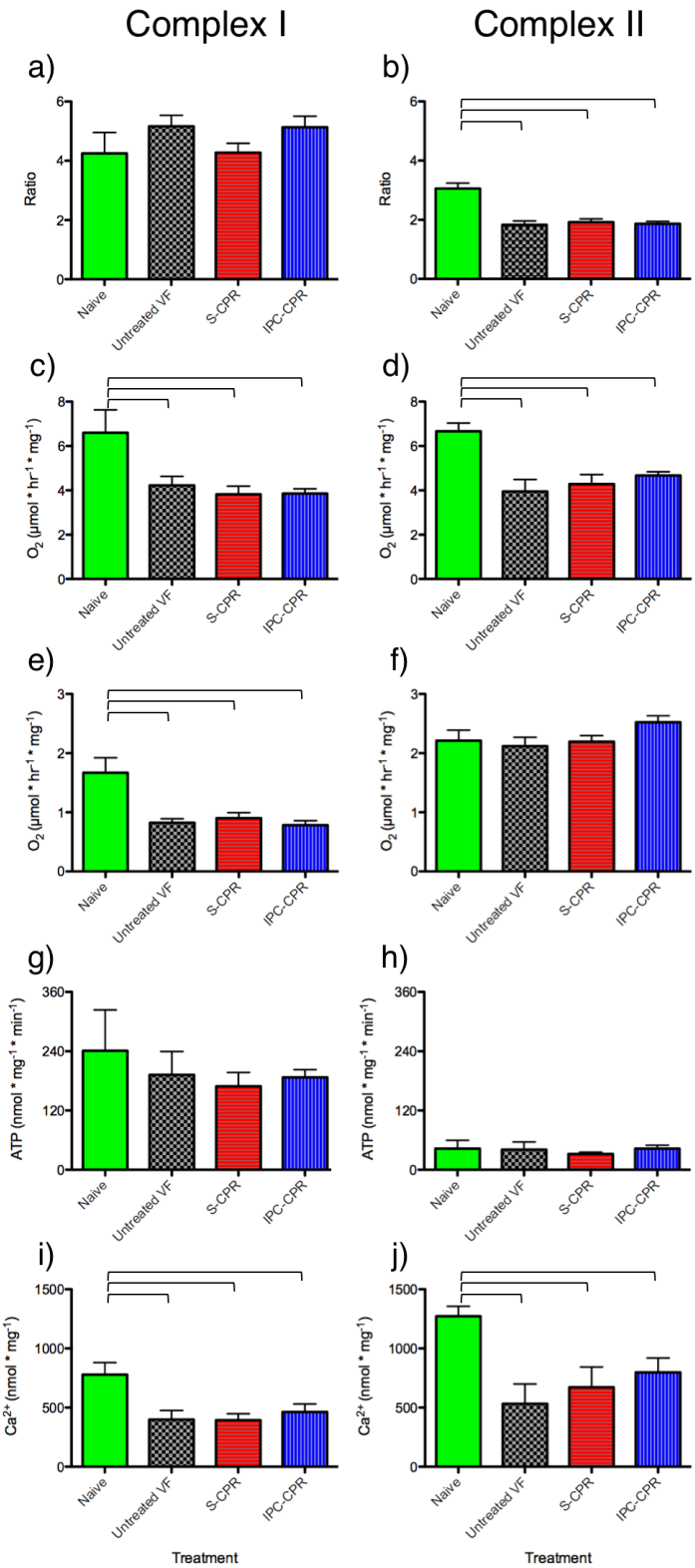

Figure 4-1.4. Cerebral mitochondrial function tests.

Figure 4-1.4. Cerebral mitochondrial function tests. Function tests were assessed with complex I substrates pyruvate and malate (left column) or complex II substrate succinate plus complex I inhibitor rotenone (right column). RCI (a, b) was calculated as the ratio of State 3 (S3; c, d) to State 4 (S4; e, f) respiration. Complex I RCI was unaffected by treatment due to proportional decreases in S3 and S4 respiration in all ischemic groups. Complex II RCI decreased in all ischemic groups compared to Naïve due to decreased S3 respiration. The rate of ATP synthesis was unchanged between treatments (g, h). CRC decreased in all ischemic groups compared to Naïve (i, j). Data are shown as mean ± SEM; brackets indicate $p < 0.05$ between groups.

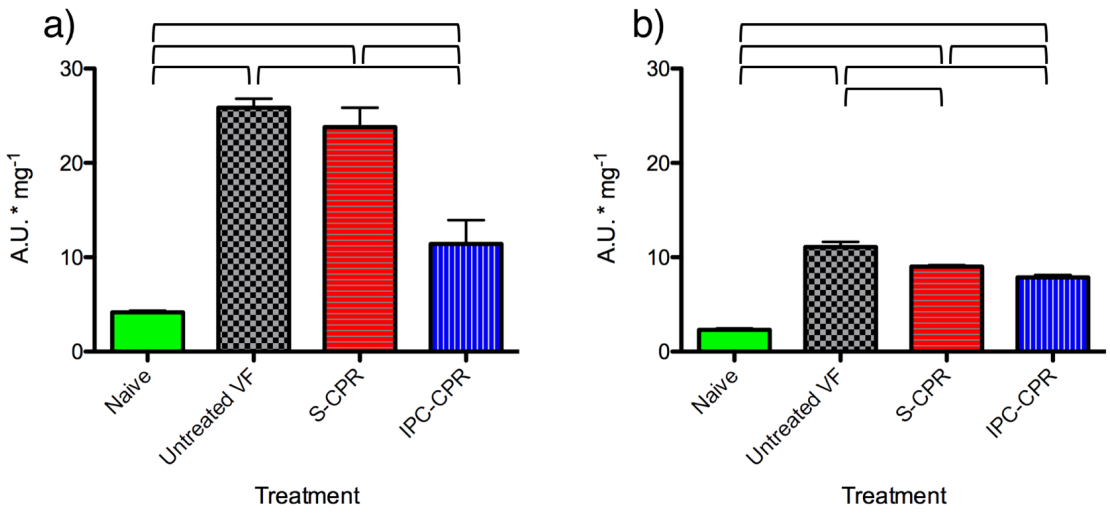

Figure 4-1.5. ESR spectra from fresh-frozen heart and brain tissue. Aconitase radical intensity for cardiac (a) and brain (b) samples. Untreated VF increased oxidized aconitase compared to Naïve treatment. Postconditioning with IPC-CPR decreased aconitase ROS production from cardiac and brain biopsies taken during the 4th minute of CPR compared to S-CPR. Data are shown as mean ± SEM; brackets indicate $p < 0.05$ between groups.

SUPPLEMENTAL METHODS

Animal Preparation

Female domestic crossbreed swine weighing 42.2±0.4 kg were sedated with 25 mg/kg ketamine IM. Animals were subsequently intubated, positive-pressure ventilated with room air (Dräger 4A, Narkomed, Telford, PA), and anesthetized with isoflurane (1.0-2.0 Vol%). Tidal volume (8-12 ml kg^{-1}) and FiO_2 were administered to maintain end-tidal CO_2 ($ETCO_2$) between 38-42 mmHg and O_2 saturation ≥ 90% (CO_2SMO Plus, Novametrix Systems, Wallingford, CT). Esophageal temperature was measured and maintained via a heating blanket (Bair Hugger, Augustine Medical, Eden Prairie, MN) at 37±0.5 °C throughout surgical preparation.

The left femoral artery and right external jugular vein were cannulated percutaneously with 8 French sheaths and micromanometer tipped catheters (Mikro-Tip Transducer, Millar Instruments, Houston, TX) were inserted to continuously measure aortic and right atrial pressures, respectively. Echocardiograms (ECG), $ETCO_2$, transcutaneous O_2 saturation, and temperature were continually recorded (Biopac Systems, Goleta, CA and LabView, National Instruments, Austin, TX). An IV bolus of heparin (5000 units) was administered once catheters were in place. An arterial blood gas was drawn after surgical preparation (Instrumentation Laboratory, Bedford, MA).

Mitochondrial Isolation

At the conclusion of the experimental protocol, mitochondria were isolated at 4 °C via differential centrifugation from the left ventricle of the heart and brain as described previously (186,187). All reagents were purchased from Sigma (St. Louis, MO).

Cardiac mitochondria: The heart was removed via lateral thoracotomy immediately after chest compression were discontinued. Three g of left ventricular tissue was placed in ice-cold isolation buffer (200 mmol mannitol, 50 mmol sucrose, 5 mmol KH_2PO_4, 5 mmol MOPS, 1 mmol EGTA, 0.1% BSA, pH adjusted with KOH to 7.15), minced, and homogenized in the presence of 5 U ml^{-1} Bacillus Licheniformis. The suspension was centrifuged for 10 min at 8,000 g. The pellet was resuspended in 25 ml isolation buffer, followed by centrifugation for 10 min at 750 g. The resulting supernatant was centrifuged

for 10 min at 8,000 g, and the pellet resuspended in 800 µl isolation buffer and stored on ice. Protein concentration was determined via the Bradford method (212).

Brain mitochondria: The brain was removed while the animal was supine with ongoing chest compressions to maintain cerebral perfusion. An incision was made in the scalp and the skull was removed with a bone saw (810 Autopsy Saw, Stryker, Kalamazoo, MI). The whole brain was removed and placed in ice-cold saline. Both hippocampi and the cerebellar vermis were combined with parietal cortex to a total of 8 g, representing a globally distributed sample of brain comprising regions sensitive to ischemia and required for executive function. This combination was placed in ice-cold isolation buffer, minced, and homogenized via Dounce-style glass homogenizer (885300-0100, Kimble Chase, Rockwood, TN). The suspension was centrifuged for 3 min at 1,330 g. The supernatant was saved, and the pellet was suspended in isolation buffer and spun at 1,330 g for 3 min. The resulting supernatants were combined and spun for 10 min at 21,000 g. The pellet was suspended in isolation buffer containing 15% Percoll (GE Healthcare Bio-Sciences, Pittsburgh, PA). This solution was layered on top of a Percoll density gradient containing 40% and 24% Percoll in isolation buffer. The gradient was spun at 30,700 g for 9 min to isolate mitochondria from synaptosomes. The mitochondrial fraction was identified as the fraction between the 24% and 40% Percoll, and was twice suspended in isolation buffer and spun at 16,900 g and 6,900 g for 10 min each to remove excess Percoll. The resulting pellet was resuspended in 400 µl isolation buffer. Protein concentration was determined via the Bradford method (212).

Mitochondrial Function Tests

Respiration: Experiments were conducted at 25 °C with mitochondria suspended (0.5 mg ml^{-1}) in experimental buffer (130 mmol KCl, 5 mmol K_2HPO_4, 5 mmol MOPS, 0.1% BSA, pH adjusted to 7.15 with KOH). Mitochondrial O_2 consumption was measured with a Clark-type O_2 electrode (Model 1302, Strathkelvin Instruments, North Lanarkshire, Scotland) in a water-jacketed 550-µl chamber (Model MT200A, Strathkelvin Instruments) monitored by an O_2 meter (Model 782, Strathkelvin Instruments). State (S) 2 respiration was initiated 60 s after sealing the chamber by adding 10 mM of the complex I substrates

pyruvate and malate or a combination of 10 mM complex II substrate succinate with 0.5 μM complex I blocker rotenone. Addition of 250 μM adenosine diphosphate (ADP) at 150 s initiated S3 respiration, until complete phosphorylation of ADP to ATP led to S4 respiration (Fig. 4S1a). Chamber O_2 concentration in μM was monitored for 60 s after S4 respiration was achieved or until the O_2 concentration was zero. Respiratory control index (RCI), a measure of the coupling of oxygen consumption to ATP generation, was calculated as the ratio of the rate of S3 to S4 respirations. All individual results are the average of duplicate runs.

ATP synthesis: The rate of mitochondrial ATP synthesis was determined by chemiluminescence (Glomax 20/20, Promega, Madison, WI) utilizing the reaction of firefly luciferase and luciferin with ATP. Each reaction contained 30 μM ADP, 10 μg ml^{-1} mitochondria, 0.1 mg ml^{-1} luciferin, and 1.25 μg ml^{-1} luciferase dissolved in experimental buffer. The reaction was measured for 100 s after addition of either 5 mM pyruvate and malate or 5 mM succinate. To obtain background activity, each measurement is repeated in the presence of ATP synthase inhibitor oligomycin (1 μg ml^{-1}). Data from measurements in the presence of oligomycin are subtracted from those obtained in the absence of oligomycin. A standard curve was obtained from known ATP concentrations to calculate the rate of ATP synthesis.

CRC: Mitochondria were suspended (0.5 mg ml^{-1}) in experimental buffer inside a cuvette-based spectrofluorometer (QuantaMaster 800, Photon Technology International, Edison, NJ) containing 10 mM of the complex I substrates pyruvate and malate or 10 mM of the complex II substrate succinate with 0.5 μM of the complex I blocker rotenone (Fig. S1b). Extramitochondrial calcium concentration ($[Ca^{2+}]_{em}$) was monitored using the fluorescent probe CaGreen-5N hexapotassium salt (C3737, Life Technologies, Carlsbad, CA) at excitation and emission wavelengths of 510 and 531 nm, respectively. After a 1-min stabilization period, $CaCl_2$ (5 mM) was infused at a rate of 30 μl min^{-1} until $[Ca^{2+}]_{em}$ reached a steady state (equilibrium between Ca^{2+} infusion and mitochondrial Ca^{2+} uptake). $CaCl_2$ was continuously infused until a rapid increase in $[Ca^{2+}]_{em}$ was observed indicating release of mitochondrial Ca^{2+} from opening of the mitochondrial permeability transition pore (mPTP). CRC was quantified as the amount of $CaCl_2$ infused until mPTP opening.

Electron Spin Resonance

Production of reactive oxygen species (ROS) was assessed with electron spin resonance (ESR) in tissue frozen in liquid nitrogen immediately upon harvest. ROS species such as superoxide and hydrogen peroxide react with mitochondrial Fe_4-S_4 clusters contributing to aconitase inactivation and resulting in a characteristic aconitase radical with g-factor 2.002 (198). Production of ROS was quantified by accumulation of aconitase free radical measured by ESR in frozen tissue using quartz Dewar and liquid nitrogen. The ESR spectrum of aconitase radical partially overlaps with other radical species. Subtraction of background ESR spectra revealed a clear aconitase free radical ESR spectrum (Fig. 4S1g). The position of this spectrum (2.002 g-factor), line-width (8 Gauss), and high temperature and microwave power dependence confirmed identification as aconitase radical (213). The ESR signals of tissue background bioradicals did not change significantly with treatments (Fig. 4S1c-f). ESR spectra were recorded (EMX ESR spectrometer, Bruker Biospin Corp., Billerica, MA) with super high Q microwave cavity (Suprasil Nitrogen Dewar Flask, Wilmad-Labglass, Vineland, NJ). The ESR settings were as follows: field sweep, 400 Gauss; microwave frequency, 9.43 GHz; microwave power, 20 milliwatts; modulation amplitude, 5 Gauss; sweep time, 80 seconds.

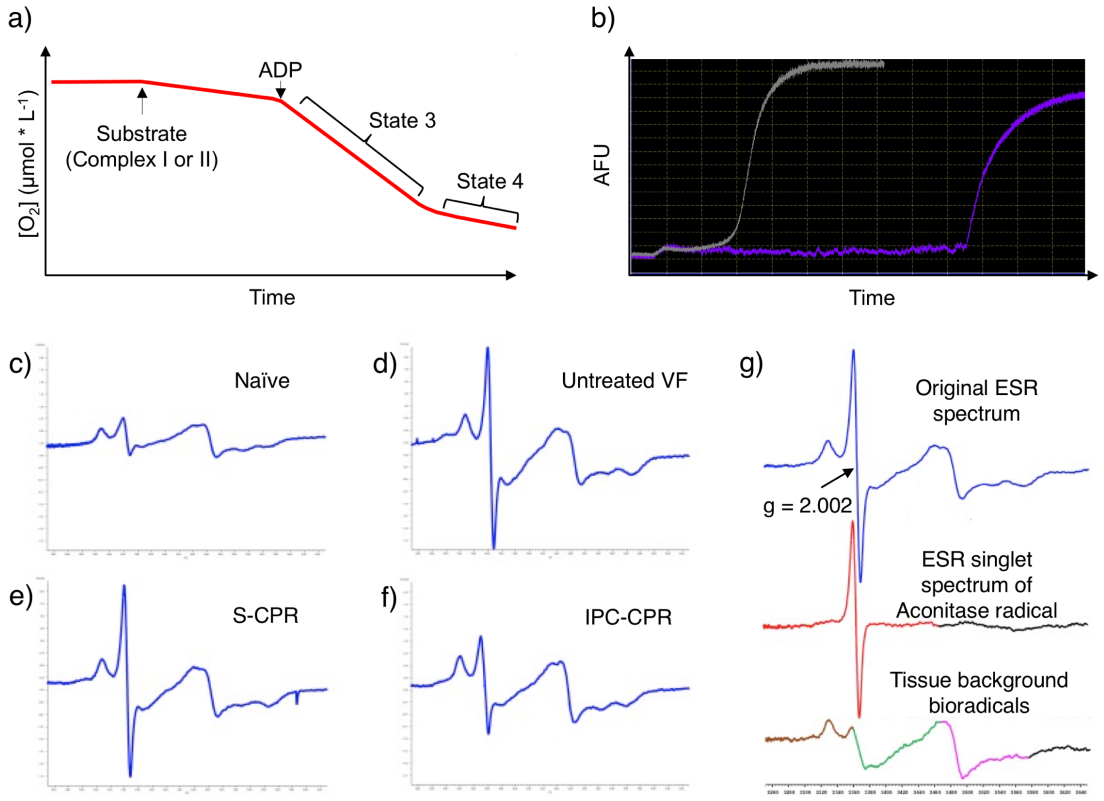

Figure 4S1. Mitochondrial function tests. Representative tracings from closed-cell respirometry (a), calcium retention capacity (CRC, b), and electron spin resonance (ESR, c-f). Respiratory control index was calculated as the ratio of State 3 to State 4 respiration. CRC was quantified as the amount of calcium added to the reaction cuvette prior to the steepest slope of extramitochondrial calcium fluorescence. The amplitude of oxidized aconitase was determined after subtracting background bioradicals from ESR spectra (g).

CHAPTER 4

Part 2

Ischemic Postconditioning Transiently Improves Mitochondrial Function during Resuscitation in a Porcine Model of Cardiac Arrest

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Synopsis

Introduction: Ischemic postconditioning (IPC) during cardiopulmonary resuscitation (IPC-CPR) has been shown to increase cardiac and cerebral mitochondrial function prior to return of spontaneous circulation (ROSC) compared to standard CPR (S-CPR) in a porcine model of ventricular fibrillation (VF) cardiac arrest (CA). IPC-CPR also reduced adverse events and improved neurologically favorable survival up to 48 hours after ROSC.

Objective: We investigated the effect of IPC-CPR after prolonged VF CA on cardiac and cerebral mitochondrial function 6 hours after ROSC.

Methods: Methods have been previously described. Briefly, 24 female swine were intubated, anesthetized, and surgically prepared for continuous hemodynamic recording. Eight animals received no CA (Naïve, n=8) as a non-ischemic control. The remaining animals received 15 minutes of untreated VF CA followed by CPR without (S-CPR, n=8) or with IPC (IPC-CPR, n=8). IPC was administered via 3 cycles of 20 seconds compressions and 20 seconds pause in chest compressions during the first 2 minutes of CPR. After 4 minutes of CPR, defibrillation was attempted. Six hours after ROSC, cardiac and cerebral mitochondria were isolated via differential centrifugation for assessment of respiration, rate of ATP synthesis, and calcium retention capacity (Fig 4-2.1).

Results: ROSC was obtained in all animals that received a CA. There were no differences in cardiac and cerebral mitochondrial function between Naïve, S-CPR, and IPC-CPR treated animals (Fig 4-2.2).

Conclusions: In a porcine model of prolonged VF CA, cardiac and cerebral mitochondrial function normalizes within 6 hours of ROSC. The previously documented reduction in adverse events and improvement in survival with IPC-CPR treatment may be mediated by acute improvements in cardiac mitochondrial function earlier than 6 hours post-ROSC, or by mechanisms independent of mitochondrial function.

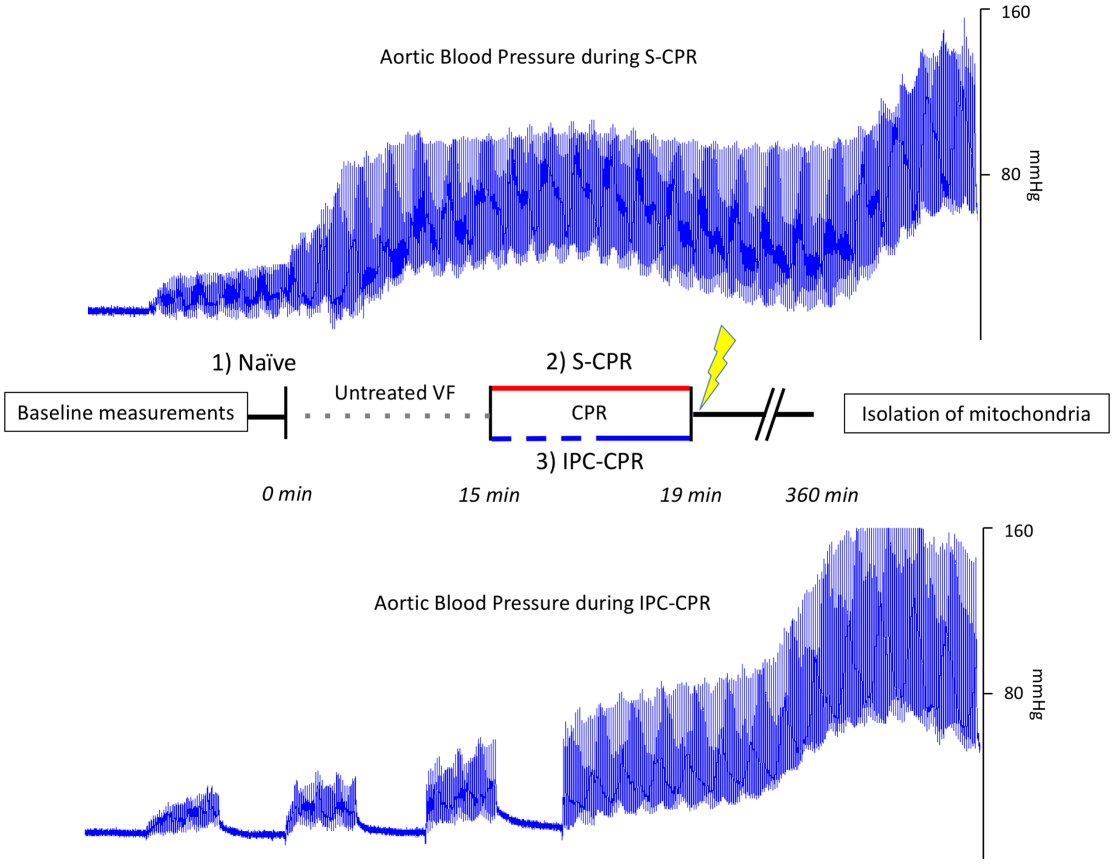

Figure 4-2.1. Experimental protocol for CPR and six-hour recovery. Animals were randomized to receive no ischemia (Naïve), standard CPR (S-CPR), or IPC at the initiation of CPR (IPC-CPR). After 4 minutes of CPR, defibrillation was attempted. If return of spontaneous circulation was achieved, animals were recovered for 6 hours prior to isolation of mitochondria from the heart and brain for function testing.

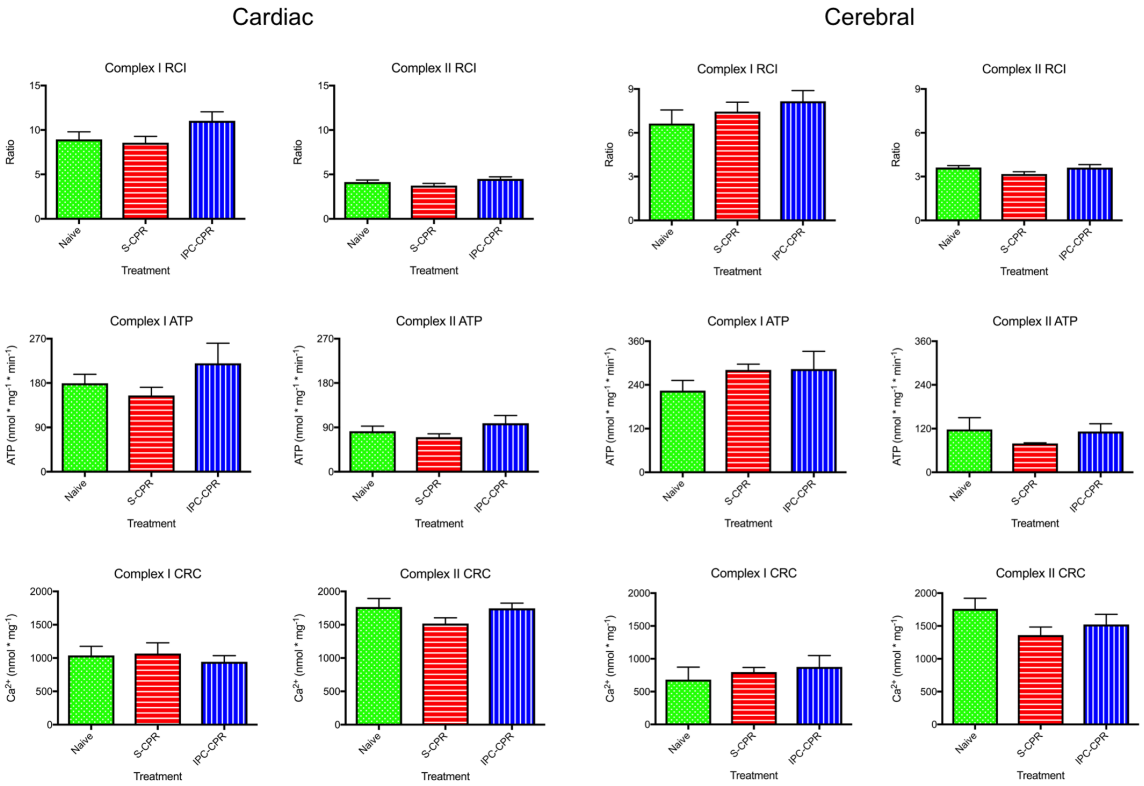

Figure 4-2.2. Cardiac and cerebral mitochondrial function 6 hours after ROSC. Cardiac (left two columns) and cerebral (right two columns) mitochondrial function did not differ between treatments. Function was assessed with complex I substrates pyruvate and malate or complex II substrate succinate plus complex I inhibitor rotenone. RCI = respiratory control index calculated as the ratio of State 3 to State 4 respiration; ATP = the rate of ATP synthesis; CRC = calcium retention capacity. Data are shown as mean ± SEM; brackets indicate $p < 0.05$ between groups.

CHAPTER 5

Coronary Perfusion Pressure Mediates Cardiac Mitochondrial Function during Resuscitation with Ischemic Postconditioning in a Porcine Model of Prolonged Cardiac Arrest

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Synopsis

Introduction: Ischemic postconditioning (IPC) has been shown to improve cardiac mitochondrial respiration during CPR as well as left ventricular ejection fraction post-ROSC in a porcine model of prolonged cardiac arrest compared to standard CPR. IPC during CPR (IPC-CPR) also increased coronary perfusion pressure (CPP) after bolus IV epinephrine. We investigated if increased CPP obtained during resuscitation with IPC-CPR is necessary and sufficient to improve cardiac mitochondrial respiration. In Protocol 1, CPP during IPC-CPR was decreased to that of standard CPR (S-CPR) by: a) withholding epinephrine during CPR, and b) controlling CPP to <30mmHg via shallow chest compressions. In Protocol 2, resuscitation with extracorporeal membrane oxygenation (ECMO) was implemented to provide high CPP during standard continuous ECMO (S-ECMO) or with IPC during ECMO (IPC-ECMO).

Methods: All animals were intubated, anesthetized with isoflurane, and received 15 minutes of ventricular fibrillation (VF). In Protocol 1, 30 female pigs were randomized to receive S-CPR (n=8), IPC-CPR (n=8), IPC without epinephrine (IPC-noEpi, n=6), or IPC with controlled CPP (IPC-CTL CPP, n=8). In Protocol 2, 14 female pigs were randomized to receive venous-arterial ECMO with continuous flow (S-ECMO, n=7) or with IPC (IPC-ECMO, n=7). All groups in both protocols, except IPC-noEpi, received 0.5 mg of epinephrine at minute 3 of CPR. IPC consisted of 3 cycles of 20 seconds compression / 20 seconds pause for the first 2 minutes of CPR or ECMO. Central arterial and venous pressures were continuously measured during resuscitation. CPP was calculated as the difference between arterial and venous pressure during the decompression phase of CPR or continuously during ECMO. Cardiac mitochondria were isolated via differential centrifugation 4 minutes after initiation of CPR or ECMO reperfusion. Additionally, a Doppler flow probe was surgically placed on the left anterior descending (LAD) coronary artery of animals receiving ECMO. Respiration, rate of ATP synthesis, and calcium retention capacity (CRC) were measured for complex I (pyruvate+malate) and II (succinate+rotenone) substrates. Respiratory control index (RCI) was calculated as the ratio of states 3 to 4 respiration. Data were compared between CPR treatment groups with ANOVA and between ECMO groups with unpaired, 2-tailed t-test.

Results: Protocol 1: CPP during the 4th minute of CPR and complex I RCI were higher in the IPC-CPR group compared to all other groups. IPC-CPR also trended toward higher complex II RCI compared to S-CPR and IPC-noEpi, but not compared to IPC-CTL CPP. CRC was not different between groups. Protocol 2: CPP, LAD coronary blood flow, and mitochondrial function did not differ between S-ECMO and IPC-ECMO.
Conclusion: IPC-CPR with high-quality chest compressions and epinephrine increased CPP and cardiac mitochondrial respiration compared to other CPR groups. Resuscitation with ECMO provided high CPP similar to IPC-CPR. There were no differences in perfusion pressure, coronary flow, or mitochondrial function between S-ECMO and IPC-ECMO. These findings suggest that elevated CPP during resuscitation is necessary and sufficient for salvage of cardiac mitochondrial function after prolonged cardiac arrest. Prior observations of improved post-resuscitation organ function and survival conferred by IPC-CPR may be mediated, in part, by improved hemodynamics during resuscitation.

Introduction

Outcomes after out-of-hospital cardiac arrest remain poor despite recent advances in care (2). Survival is negatively correlated with time of untreated ischemia, which has led to a 3-phase theoretical model for understanding the increasing severe pathophysiology during cardiac arrest (214). The model predicts that for cardiac arrests lasting more than 10 minutes, termed the metabolic phase, reperfusion injury and metabolic derangement are principle factors preventing successful resuscitation. Further, the model predicts that targeting the underlying pathology of the arrest with the initial resuscitation efforts will yield the greatest improvement in outcomes.

IPC is a technique of administering short, non-lethal bouts of ischemia during early reperfusion after prolonged ischemia to mitigate reperfusion injury (11). When applied at the onset of perfusion with CPR, IPC increased CPP after exogenous epinephrine, decreased post-ROSC cardiac dysfunction, and increased acute cardiac mitochondrial function as well as hemodynamics during resuscitation after exogenous epinephrine (74,215).

The objective of these studies was to determine if elevated CPP during resuscitation

is necessary and sufficient to restore cardiac mitochondrial function following prolonged untreated ventricular fibrillation cardiac arrest in a porcine model.

Methods

Methods have been described previously (215). Briefly, female Yorkshire domestic crossbreed swine were intubated, anesthetized, and prepared for continuous ECG and blood pressure monitoring. In Protocol 2, a Doppler flow probe (Transonic Systems, Ithaca, NY) was placed around the LAD coronary artery via midline thoracotomy. In both protocols, ventricular fibrillation was induced via temporary pacing wire and cardiac arrest was left untreated for 15 minutes. CPP was calculated as the difference between arterial and venous pressures during the decompression phase of CPR or continuously during ECMO. CPP and LAD blood flow were used to determine LAD coronary vascular resistance. IPC was administered via 3 cycles of 20 seconds perfusion and 20 seconds pause for the first 2 min of CPR or ECMO resuscitation.

Protocol 1 - CPR was administered as previously described for S-CPR (n=8) and IPC-CPR groups (n=8) (215). The IPC-noEpi group (n=6) did not receive a bolus dose of epinephrine during resuscitation. The IPC-CTL CPP group received incomplete chest compression depth to target a CPP value of 30 mmHg (Fig 5.1).

Protocol 2 – Venous-Arterial ECMO (Maquet Cardiohelp, Rastatt, Germany) perfusion was administered via a 21-F venous cannula placed in the inferior venacava at the level of the right atrium and a 15-F arterial cannula placed in the femoral artery. Animals were randomized to receive S-ECMO (n=7) or IPC-ECMO (n=7).

In both protocols, 3 g of left ventricle was obtained after 4 minutes of reperfusion and cardiac mitochondria were immediately isolated via differential centrifugation for function assays as previously described (215).

Results

Protocol 1

CPP during the 4th minute of CPR was higher in the IPC-CPR group than S-CPR, IPC-noEpi, and IPC-CTL CPP (Fig 5.2). Complex I RCI was higher in the IPC-CPR group

compared to all other groups (Fig 5.3). IPC-CPR also trended toward higher complex II RCI compared to S-CPR and IPC-noEpi, but not compared to IPC-CTL CPP. CRC was not different between groups.

Protocol 2

S-ECMO and IPC-ECMO obtained CPPs similar to IPC-CPR. There were no differences between ECMO groups in CPP, LAD blood flow, LAD vascular resistance, or mitochondrial function (Fig 5.4).

Discussion

Increasing blood flow during CPR has been a primary focus of resuscitation science for decades. However, IPC-CPR is a therapeutic strategy intended to mitigate reperfusion injury, and was anticipated to do so at the expense of blood flow during resuscitation. Contrary to predicted outcome, IPC-CPR had the effect of increasing arterial blood pressure and calculated CPP during CPR after administration of exogenous epinephrine (74,75,183,215). Poloxamer 188, a component of the Bundle postconditioning, independently increased blood pressure when administered during CPR (Fig 5.5) (216). Thus, hemodynamic stability may contribute to the therapeutic value of IPC-CPR in addition to canonical postconditioning pathways (18). In this investigation, we sought to determine if the hemodynamic improvement following controlled pauses is a mechanism through which IPC-CPR confers protection to cardiac mitochondrial function following prolonged cardiac arrest.

To determine the contribution of elevated CPP on improvement of acute cardiac mitochondrial function, perfusion pressures during the 4th minute of IPC-CPR were attenuated to the level of S-CPR via 1) withholding the epinephrine bolus at minute 3 of CPR, or 2) incomplete chest compressions. In both groups, lowering CPP to the level of S-CPR resulted in decreased cardiac mitochondrial respiration compared to IPC-CPR with epinephrine and high-quality chest compressions. This finding strongly suggests a role for improved coronary perfusion as a mechanism for cardiac mitochondrial salvage provided by IPC-CPR after cardiac arrest.

Despite high-quality mechanical CPR utilizing active compression-decompression

and an impedance threshold device, CPP during S-CPR could not be elevated to that of IPC-CPR during the 4th minute of resuscitation. Consequently, we utilized ECMO to provide CPP comparable to the IPC-CPR group. We further evaluated LAD blood flow to better elucidate perfusion to the myocardium during ECMO perfusion. There were no differences between S-ECMO and IPC-ECMO in CPP, LAD blood flow, or LAD vascular resistance, suggesting CPP is a faithful indicator of cardiac perfusion during ECMO. There were no differences in cardiac mitochondrial respiration, ATP synthesis, or CRC between S-ECMO or IPC-ECMO. High cardiac perfusion was sufficient to protect cardiac mitochondrial function after prolonged cardiac arrest. It remains to be tested if non-pulsatile ECMO flow directly affects mitochondrial function or modulates the effect of IPC.

Conclusion

Cardiac mitochondrial respiration was improved with IPC when administered during high-quality CPR with epinephrine compared to CPR groups with lower coronary perfusion pressure. IPC did not provide an additional benefit to cardiac mitochondrial function when applied during high-pressure perfusion with ECMO. Taken together, these data indicate that high coronary perfusion pressure during resuscitation is necessary and sufficient to rescue cardiac mitochondrial function after prolonged cardiac arrest. Improved hemodynamics during CPR may mediate the beneficial cardiac recovery previously observed with IPC-CPR.

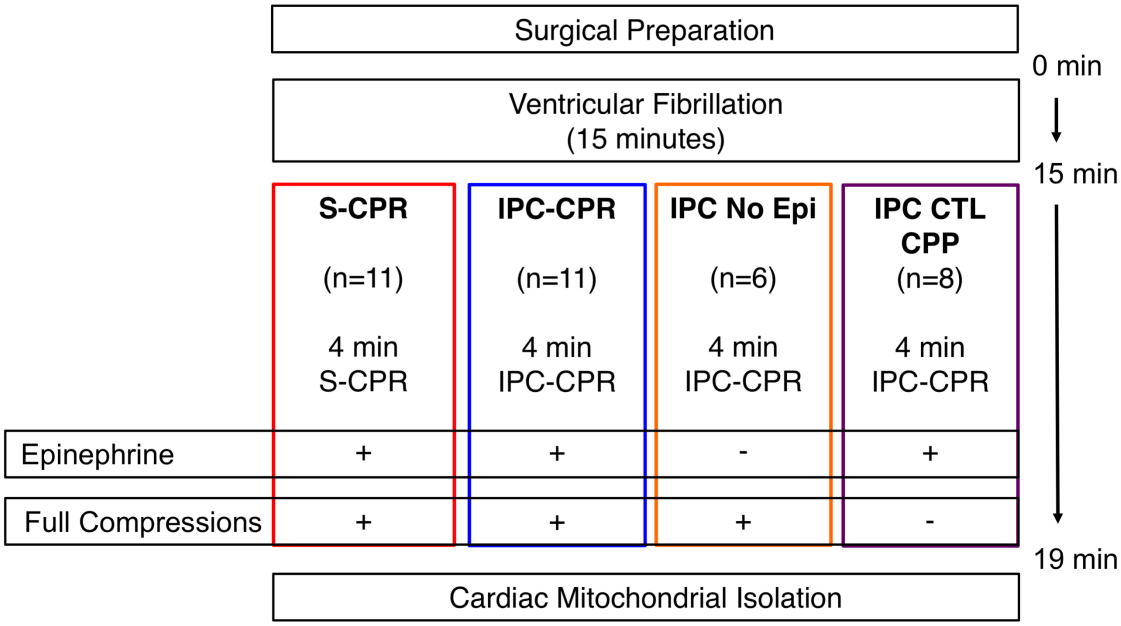

Figure 5.1. Experimental protocol for standard CPR and IPC subgroups. High-quality CPR with epinephrine served as a control for standard CPR (S-CPR). Cardiac mitochondria were isolated from left ventricle during CPR prior to defibrillation or return of spontaneous circulation.

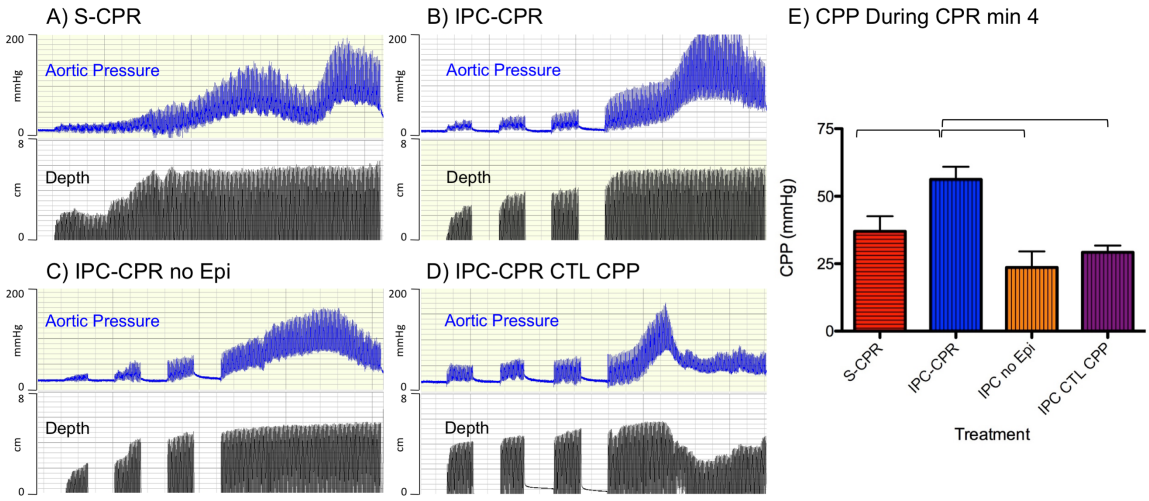

Figure 5.2. Coronary perfusion pressure during S-CPR and IPC-CPR, IPC-CPR without epinephrine, and IPC-CPR with incomplete depth of chest compression. Representative tracing of aortic blood pressure and chest compression depth during S-CPR (A), IPC-CPR (B), IPC-CPR noEpi (C), and IPC-CPR CTL CPP (D). Coronary perfusion pressure (CPP) during the 4th minute of CPR was higher in the IPC-CPR treatment than all other treatments (E). CPP during the 4th minute of CPR was similar between remaining treatments. ANOVA with Newman-Keuls posthoc, $p < 0.05$ considered significant.

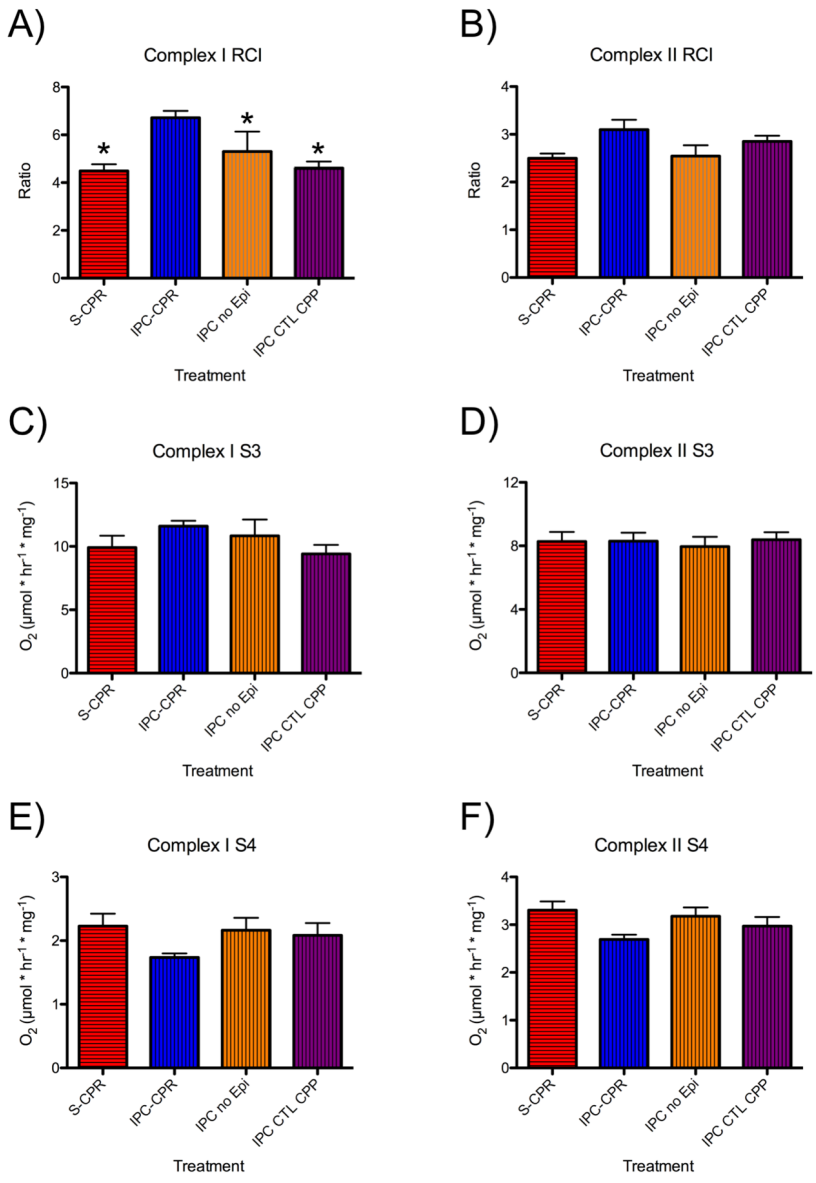

Figure 5.3. Cardiac mitochondrial respiration during standard CPR and IPC subgroups. S-CPR = high-quality standard CPR; IPC-CPR = high-quality CPR with IPC; IPC no Epi = IPC-CPR without exogenous epinephrine; IPC-CTL CPP = IPC with incomplete chest compressions; RCI = respiratory control index; S3 = ADP-stimulated state 3 respiration; S4 = state 4 leak respiration; Complex I respiration stimulated with pyruvate and malate; Complex II respiration stimulated with succinate and complex I inhibitor rotenone. Comparisons made with ANOVA with Newman-Keuls posthoc test.
* indicates $p < 0.05$ compared to IPC-CPR.

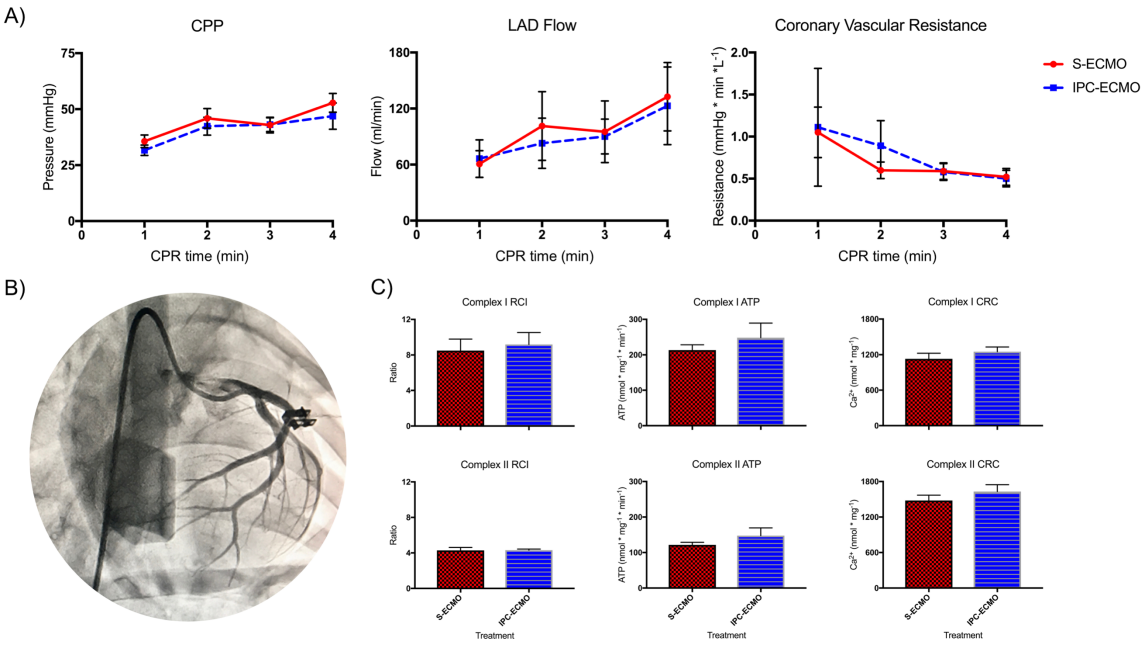

Figure 5.4. Hemodynamics and mitochondrial function during ECMO resuscitation. Resuscitation with extracorporeal membrane oxygenation (ECMO) resulted in similar cardiac perfusion between treatments (A) as evidenced by coronary pressure (CPP), left anterior descending coronary artery blood flow (LAD Flow), and LAD coronary vascular resistance. Angiographic confirmation (B) of Doppler flow probe placement on proximal LAD coronary artery. Mitochondrial function (C) did not differ between treatment groups. RCI = respiratory control index; ATP = rate of ATP synthesis; CRC = calcium retention capacity. Comparisons made with t-test, alpha 0.05.

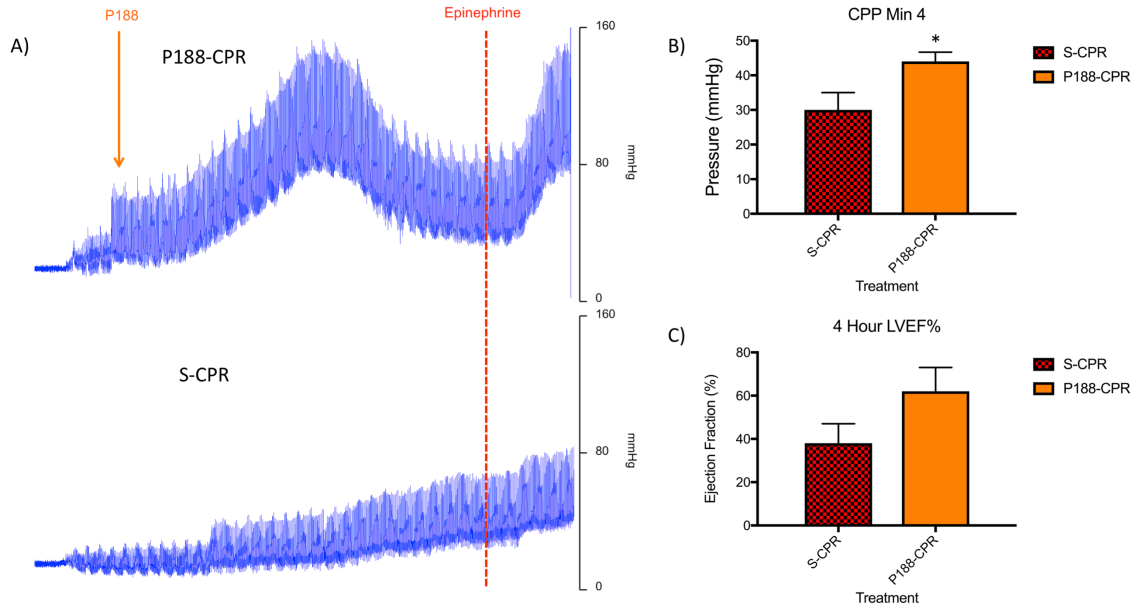

Figure 5.5. Hemodynamics and cardiac recovery during CPR with P188. Representative aortic pressure tracings from CPR with bolus dose of P188 (P188-CPR) or standard CPR (S-CPR) depicting the time of P188 (250 mg/kg) and epinephrine (0.5 mg) bolus dose administration (A). P188-CPR resulted in higher mean CPP during the 4th minute of CPR (B) and trended toward increased left ventricular ejection fraction (LVEF%) after resuscitation. N = 6 in each group; * indicates $p < 0.05$ with unpaired t-test. Adapted from Matsuura et al. Circulation 2013;128:A282.

CHAPTER 6

SUMMARY

The central hypothesis of this work is that ischemic postconditioning implemented at the initiation of CPR can mitigate reperfusion injury that represents the primary impedance to resuscitation after prolonged cardiac arrest. This work details experiments investigating the feasibility and efficacy of implementing a simple IPC strategy at the onset of CPR to mitigate mitochondrial, organ, and whole-animal injury following prolonged ventricular fibrillation cardiac arrest.

Major Findings

• IPC via controlled pauses at the initiation of CPR mitigated cardiac dysfunction and improved neurologically favorable recovery after 15 minutes of VF cardiac arrest.

• IPC alone or in combination with vasodilator therapy significantly improved clinical and histopathologic indicators of markers of cardiac and cerebral recovery. Vasodilation therapy offered an independent increase in post-ROSC cardiac function and a decrease in biomarkers, but did not provide independent or synergistic benefit to favorable neural and survival outcomes.

• A combination of IPC, sevoflurane, and P188 postconditioning improved cardiac and neurologic recovery after 17 minutes of untreated cardiac arrest demonstrated by decreased cardiac biomarkers of ischemia, increased left ventricular ejection fraction, and increased 48-hour survival with neurologically favorable outcome. This represents the longest duration of untreated cardiac arrest for which favorable neurological survival has been documented in a swine model of cardiac arrest.

• Prolonged untreated VF cardiac arrest results in acute cardiac and brain mitochondrial dysfunction evidenced by uncoupled respiration, decreased rate of ATP synthesis, decreased calcium retention capacity, and increased ROS production. IPC-CPR attenuated cardiac mitochondrial dysfunction, but had limited effect on brain mitochondria. The

benefit of IPC-CPR to neural recovery is likely mediated by a different mechanism or on a longer temporal scale.

• Cardiac mitochondrial function normalized to non-ischemic levels within six hours of return of spontaneous circulation in animals treated with either S-CPR or IPC-CPR.

• IPC increased cardiac mitochondrial respiration after prolonged cardiac arrest only in the setting of high-quality mechanical CPR with epinephrine. Attenuating the normal increase in coronary perfusion pressure during IPC-CPR abrogated the increase in cardiac mitochondrial function previously observed. CPP and mitochondrial function were comparable to S-CPR, indicating that increased perfusion pressure during IPC-CPR mediates improvements in cardiac mitochondrial function.

• High-quality perfusion with ECMO provides salvage of cardiac mitochondrial respiration after prolonged cardiac arrest. In this setting, administration of IPC during ECMO perfusion did not provide further benefit to acute cardiac mitochondrial function.

Perfusion versus reperfusion injury

Sodium nitroprusside (SNP) enhanced CPR (SNPeCPR) represents the height of pharmacologic and mechanical means to generate blood flow during CPR. In addition to increased blood flow, SNP may itself offer postconditioning benefits via NO donation (87,217). If SNPeCPR does indeed confer some of its benefit via attenuation of reperfusion injury, then the only therapeutic strategies shown to successfully resuscitate swine after 15 minutes of ventricular fibrillation cardiac arrest have involved both postconditioning strategies and strong hemodynamic support (7,74,75,84,122,183). Recently, superior perfusion with SNPeCPR demonstrated a short-term survival benefit in a novel model of refractory VF (218). These findings indicate that survival is positively correlated with both intra-CPR perfusion and with postconditioning during CPR, making it difficult to parse which therapeutic modality is responsible for the observed benefits.

We have also shown that improved cardiac mitochondrial function during IPC-CPR is dependent on increased CPP. Diminishing the increase in CPP afforded by IPC-CPR abrogated the improvement in acute cardiac mitochondrial function. High-quality perfusion with ECMO salvaged cardiac mitochondrial function to a similar extent as IPC-

CPR or IPC-ECMO. These observations may be critical for understanding the unique utility of postconditioning in the setting of cardiac arrest to simultaneously reverse hemodynamic stasis and mitigate reperfusion injury. Canonical cellular protection and improved blood flow may both be at work, thereby addressing multiple limitations to successful resuscitation. A multifocal mechanism may increase the therapeutic value of IPC to benefit a diverse patient population with multiple comorbidities and varying durations of untreated ischemia, though this potential has yet to be tested.

Our results indicate that increasing blood pressure and blood flow during CPR is beneficial. This is true even in the combined postconditioning group that attained hypertensive pressures during CPR (183). Our data agree with previous findings that blood pressure and blood flow during CPR positively correlate with outcomes (219,220). If reperfusion injury were the primary factor limiting resuscitation after prolonged cardiac arrest, hemodynamics would not be expected to so robustly predict outcomes. Thus, restoring adequate vital organ blood flow should remain a primary goal of resuscitation techniques.

Differences between heart and brain recovery

Cerebral blood flow was not measured in these studies. However, cerebral perfusion relies on central arterial pressures and may be assumed to positively correlate with central blood pressure under similar treatment conditions. Increased aortic blood pressure during IPC-CPR may contribute to improved cerebral perfusion and thus to neural recovery. However, enhanced neural recovery occurred independently of improved hemodynamics when IPC was compared to vasodilator therapy (75). These findings suggest an undetermined mechanism for neural protection conferred by IPC-CPR that may not solely be dependent on blood pressure during CPR.

In this series of studies, IPC-CPR conferred a benefit to neural recovery when measured at 24 and 48 hours as evidenced by decreased neuronal cell death and improved cerebral performance category. However, cerebral recovery was not observed via mitochondrial function tests measured during resuscitation or at 6 hours after ROSC. This may indicate that mitochondrial function does not mediate neural recovery following IPC-

CPR. Alternatively, this may simply demonstrate that neuronal fate is not immediately determined. Both neuronal injury and recovery likely continue to develop for hours and days after cardiac arrest (221,222). Further studies are necessary to better define the type and timing of injury specific to the vital organs of the heart and brain.

Global postconditioning response

Dynamic blood pressure response to exogenous epinephrine during IPC-CPR, the initial observation that prompted investigations in Chapter 5, may be evidence of a global postconditioning benefit. Following the IPC protocol administered in the first two minutes of reperfusion, the vasculature is conditioned to respond more dynamically to exogenous epinephrine. This phenomenon is indicative of global postconditioning perhaps targeting endothelial cells or vascular smooth muscle. Initial IPC experiments of myocardial ischemia demonstrated improved coronary vascular reactivity (11), and increased blood pressure response to epinephrine is associated with successful resuscitation (223). In the setting of cardiac arrest, increased blood pressure response to exogenous epinephrine may be a bioassay demonstrating the health of the animal in addition to mediating vital organ perfusion and recovery. Epinephrine by itself may provide additional conditioning via alpha adrenergic receptors (211,224). However, epinephrine does not appear to be necessary for IPC-CPR to protect the heart and brain. IPC demonstrated a survival benefit when administered without epinephrine in a basic life support (BLS) model of CPR (225). Even absent epinephrine in this BLS model, IPC increased perfusion pressures compared to BLS CPR alone. Additional studies to elucidate the direct effect of IPC-CPR on vascular function are warranted.

Ongoing investigations

The canonical postconditioning mechanisms of reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) will be evaluated in heart and brain samples from the experiments described. The combined results from blood pressure manipulation during resuscitation and from biochemical characterization of RISK and SAFE pathways will better elucidate the roles of blood flow and postconditioning in

mediating the cardiac and neural benefits of IPC-CPR.

Evidence of mitochondrial permeability transition pore opening in vivo will be evaluated via quantification of cytochrome c translocation from mitochondrial to cytosolic cellular fractions. Circulating levels of cytochrome c have been shown to correlate with survival after cardiac arrest (226,227). Evaluating the extent of cytochrome c release in the heart and brain would elucidate a connection between mitochondrial dysfunction and organ recovery. Transmission electron microscopy of fixed heart and brain tissue will be evaluated for mitochondrial swelling, cristae disorganization, and density (228). These morphological assessments may better describe the type of stress experienced in vivo and aid in the interpretation of mitochondrial function tests.

Cardiac mitochondrial function was transiently increased with IPC-CPR compared to S-CPR. However, cardiac mitochondrial function normalized in both groups after 6 hours of recovery. The effect of the early window of mitochondrial functional recovery remains to be fully measured. The extent of mitochondrial-mediated apoptosis, which may be initiated early and manifest late, was not measured in the 6-hour recovery studies. Preliminary results indicate minimal cell death in the heart and brain with either S-CPR or IPC-CPR at 6 hours. TUNEL staining and blotting for caspase and calpain activity will be utilized to further identify the extent of apoptosis and necrosis in the heart and brain.

Limitations

Many limitations have been named in the preceding chapters. Of overarching relevance, it remains unknown if IPC protects the heart and brain via improved hemodynamics or via mitigation of reperfusion injury. A full characterization of intracellular signaling will be required to complement the completed hemodynamic investigation to better describe the relative contributions of each.

The mechanisms via which IPC-CPR improved cardiac mitochondrial function as well as favorable cardiac and neural recovery were not determined. We selected mitochondrial function assays as a starting point because a) we predicted mitochondrial function would change dynamically within the time course of interest, and would therefore represent an upstream regulator of many protective and pathological processes; b)

mitochondrial function must recover for successful resuscitation, and therefore are a critical component for understanding limitations to resuscitation after prolonged cardiac arrest; and c) mitochondria are a nexus for many signals, and therefore represented a likely candidate to begin unraveling treatment differences. This work has begun laying foundational knowledge and techniques to better parse specific interactions. Reactive oxygen species, shown in our model to be increased during untreated cardiac arrest and S-CPR and attenuated with IPC-CPR, are a potential candidate for future study. ROS are a component of cellular stress during ischemia and reperfusion, and are also implicated in mediating the protection of IPC (229,230). Levels of oxidized aconitase, measured via EPR, were the only conserved pattern between heart and the brain responses to reperfusion with CPR. Further corroboration of these findings can be performed by quantifying oxidized lipids and proteins in samples from the heart and brain, as well as future experiments quantifying site-specific mitochondrial ROS production in isolated myofibers and brain homogenates. Endothelial and smooth muscle signaling are also prime targets based on the consistent blood pressure signature we observed during IPC-CPR. Identifying a causal link between IPC-CPR and improved mitochondrial function, or between IPC-CPR and organ recovery, would be valuable for understanding the pathophysiology of prolonged cardiac arrest as well as elucidating targeted treatment options.

These studies do not demonstrate a causal connection between acute cardiac mitochondrial function and long term outcomes. However, we have now shown in separate cohorts that IPC-CPR improves acute mitochondrial function, post-ROSC LVEF%, and neurologically favorable survival (74,215). Additionally, the mitochondrial function tests correlated well with the extent of cardiac injury in an ST-elevation myocardial infarction model of cardiac ischemia (231). An early goal of this investigation was to identify candidate mediators of protection that could be further interrogated. To date, we have explored only the role of improved hemodynamics in mediating cardioprotection. Results from ongoing characterization of RISK and SAFE pathways may allow future exploration of mechanisms through which IPC affects mitochondrial function, or through which mitochondrial function affects organ salvage. If candidate signaling pathways are identified, inhibitors and agonists will be applied to interrogate a causal connection.

All experiments were conducted in a swine model of cardiac arrest. The inherent advantages of this model are similarities between swine and human cardiovascular physiology and close adherence to clinical CPR and resuscitation practices (232). The complexity of a whole-animal model may introduce unknown and uncontrolled factors that influence results. However, those unknown factors are precisely the validity of an animal model by allowing a complex biological system to function independently of all but a few experimental treatments. It may be an advantage that only the most robust treatment signals will manifest among many unknown variables, as this showcases the potential translation to an even more heterogeneous human population. The swine model of cardiac arrest remains a vital experimental tool to vet efficacy for human translation and to generate hypotheses for more controlled model systems. However, insights may be more efficiently determined with a balanced approach utilizing smaller model organisms, isolated organ preparations, and cell culture.

The clinical translatability of IPC-CPR is yet to be fully tested. The swine were young and healthy without comorbidities normally associated with cardiac arrest that will likely attenuate the benefit of IPC-CPR (233). Additionally, clinical implementation of an IPC strategy will be more difficult than in the controlled laboratory setting. Many attempts at translation have been made with muted success, perhaps due to experimental design, pharmacological side effects, and protocol limitations (234–236). Unfortunately, IPC efficacy may be limited in the most severely diseased patients for whom its benefits are most needed. Use of disease models would increase understanding of specific mechanisms of IPC and clarify translation potential. Also of importance, IPC-CPR will require rescuers trained in advanced resuscitation techniques or the use of mechanical chest compression devices. The benefit of IPC-CPR may be abrogated by initial perfusion via bystanders prior to arrival of advanced care. The benefit of IPC-CPR has not been investigated in shorter intervals of untreated cardiac arrest. Additional investigations are required to properly optimize deployment of IPC-CPR in the field.

All CPR was performed as technically sound as possible. This does not represent clinical reality. Bystander CPR, resuscitation in small spaces or moving vehicles, thoracic trauma, patient dimensions, rescuer fatigue, and other factors will diminish the efficacy of

chest compressions and rescue breaths. While this limits translatability, it is critical to apply prior advancements in CPR techniques to current investigations because treatment effects can be masked by unintended protocol deviations (237). Ideally, best practice will be utilized in laboratory and clinical settings and thereby become the next standard of care.

Perspective and closing remarks

IPC drastically improved cardiac and neurologic outcomes after prolonged ventricular fibrillation cardiac arrest. IPC improved hemodynamics during CPR and increased cardiac mitochondrial function in the acute phase of resuscitation. The mechanisms that mediate cardiac and cerebral protection remain unknown. Mounting evidence suggests increased coronary perfusion pressure is necessary and sufficient for cardioprotection. Definitive evidence of reperfusion injury is yet to be established in our model due to the confounding factor that all postconditioning strategies improve hemodynamics when deployed at the onset of CPR following prolonged cardiac arrest. This work is vital for demonstrating the therapeutic potential of IPC implemented during CPR. Results from these experiments highlight the impact of early CPR interventions on long-term outcomes, and emphasize the importance of continued innovation in resuscitation therapies.

We investigated potential mechanisms responsible for the low rates of survival after prolonged cardiac arrest achieved in the field. Characterizing hemodynamic and mitochondrial functional responses during the primary stages of cardiac arrest, CPR, and post-ROSC recovery may improve understanding of the pathogenesis of lethal reperfusion injury and describe the role of IPC in preventing its progression. Results from these studies provide the groundwork for future basic science investigations into the underlying pathophysiology of cardiac arrest to optimize therapies for resuscitation after prolonged cardiac arrest. Every incremental improvement to resuscitation care has the potential to save thousands of lives.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart Disease and Stroke Statistics—2015 Update. Circulation [Internet]. 2014 Dec 18; Available from: http://circ.ahajournals.org/content/early/2014/12/18/CIR.0000000000000152.abstract
2. Medicine I of. Strategies to Improve Cardiac Arrest Survival: A Time to Act [Internet]. Graham R, McCoy MA, Schultz AM, editors. Washington, DC: The National Academies Press; 2015. Available from: https://www.nap.edu/catalog/21723/strategies-to-improve-cardiac-arrest-survival-a-time-to-act
3. Kouwenhoven WB, Jude JR, Knickerbocker GG. Closed-chest cardiac massage. JAMA. 1960 Jul;173:1064–7.
4. Halperin HR, Tsitlik JE, Guerci AD, Mellits ED, Levin HR, Shi AY, et al. Determinants of blood flow to vital organs during cardiopulmonary resuscitation in dogs. Circulation [Internet]. 1986;73(3):539–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3948359
5. Plaisance P, Lurie KG, Payen D. Inspiratory impedance during active compression-decompression cardiopulmonary resuscitation: a randomized evaluation in patients in cardiac arrest. Circulation. 2000 Mar;101(9):989–94.
6. Plaisance P, Lurie KG, Vicaut E, Adnet F, Petit JL, Epain D, et al. A comparison of standard cardiopulmonary resuscitation and active compression-decompression resuscitation for out-of-hospital cardiac arrest. French Active Compression-Decompression Cardiopulmonary Resuscitation Study Group. N Engl J Med. 1999 Aug;341(8):569–75.
7. Yannopoulos D, Matsuura T, Schultz J, Rudser K, Halperin HR, Lurie KG. Sodium nitroprusside enhanced cardiopulmonary resuscitation improves survival with good neurological function in a porcine model of prolonged cardiac arrest. Crit Care Med. 2011;39(6):1269–74.
8. Yannopoulos D, Bartos JA, Martin C, Raveendran G, Missov E, Conterato M, et al. Minnesota Resuscitation Consortium's Advanced Perfusion and Reperfusion Cardiac Life Support Strategy for Out-of-Hospital Refractory Ventricular Fibrillation. J Am Hear Assoc Cardiovasc Cerebrovasc Dis [Internet]. 2016 Jun 13;5(6):e003732. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937292/
9. Bartos JA, Voicu S, Matsuura TR, Tsangaris A, Sideris G, Oestreich BA, et al. Role of Epinephrine and Extracorporeal Membrane Oxygenation in the Management of Ischemic Refractory Ventricular Fibrillation. JACC Basic to Transl Sci [Internet]. 2017 Jun 7; Available from: http://www.basictranslational.onlinejacc.org/content/early/2017/05/31/j.jacbts.2017.02.003.abstract
10. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med. 2007

Sep;357(11):1121–35.
11. Zhao Z-Q, Corvera JS, Halkos ME, Kerendi F, Wang N-P, Guyton RA, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol. 2003 Aug;285(2):H579-88.
12. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. Nat Med. 2011;17(11):1391–401.
13. Wang J, Shen J, Gao Q, Ye Z, Yang S, Liang H, et al. Ischemic postconditioning protects against global cerebral ischemia/reperfusion-induced injury in rats. Stroke. 2008 Mar;39(3):983–90.
14. Eldaif SM, Deneve JA, Wang N-P, Jiang R, Mosunjac M, Mutrie CJ, et al. Attenuation of renal ischemia-reperfusion injury by postconditioning involves adenosine receptor and protein kinase C activation. Transpl Int. 2010 Feb;23(2):217–26.
15. Lin H-C, Lee T-K, Tsai C-C, Lai I-R, Lu K-S. Ischemic postconditioning protects liver from ischemia-reperfusion injury by modulating mitochondrial permeability transition. Transplantation. 2012 Feb;93(3):265–71.
16. Zhao Z-Q. Postconditioning in reperfusion injury: a status report. Cardiovasc drugs Ther. 2010 Jun;24(3):265–79.
17. Penna C, Perrelli M-G, Pagliaro P. Mitochondrial pathways, permeability transition pore, and redox signaling in cardioprotection: therapeutic implications. Antioxid Redox Signal. 2013 Feb;18(5):556–99.
18. Bartos JA, Debaty G, Matsuura T, Yannopoulos D. Post-conditioning to improve cardiopulmonary resuscitation. Curr Opin Crit Care. 2014;20(3):242–9.
19. Hagihara A, Hasegawa M, Abe T, Nagata T, Wakata Y, Miyazaki S. Prehospital epinephrine use and survival among patients with out-of-hospital cardiac arrest. JAMA. 2012 Mar;307(11):1161–8.
20. Aufderheide TP, Frascone RJ, Wayne MA, Mahoney BD, Swor RA, Domeier RM, et al. Standard cardiopulmonary resuscitation versus active compression-decompression cardiopulmonary resuscitation with augmentation of negative intrathoracic pressure for out-of-hospital cardiac arrest: a randomised trial. Lancet (London, England). 2011 Jan;377(9762):301–11.
21. Aufderheide TP, Nichol G, Rea TD, Brown SP, Leroux BG, Pepe PE, et al. A trial of an impedance threshold device in out-of-hospital cardiac arrest. N Engl J Med. 2011 Sep;365(9):798–806.
22. Sasson C, Rogers MAM, Dahl J, Kellermann AL. Predictors of survival from out-of-hospital cardiac arrest: a systematic review and meta-analysis. Circ Cardiovasc Qual Outcomes. 2010 Jan;3(1):63–81.
23. Auble TE, Menegazzi JJ, Paris PM. Effect of out-of-hospital defibrillation by basic life support providers on cardiac arrest mortality: a metaanalysis. Ann Emerg Med. 1995 May;25(5):642–8.
24. Stiell IG, Wells GA, Field BJ, Spaite DW, De Maio VJ, Ward R, et al. Improved out-of-hospital cardiac arrest survival through the inexpensive optimization of an existing defibrillation program: OPALS study phase II. Ontario Prehospital

Advanced Life Support. JAMA. 1999 Apr;281(13):1175–81.
25. Valenzuela TD, Roe DJ, Cretin S, Spaite DW, Larsen MP. Estimating effectiveness of cardiac arrest interventions: a logistic regression survival model. Circulation. 1997 Nov;96(10):3308–13.
26. Herlitz J, Engdahl J, Svensson L, Angquist K-A, Young M, Holmberg S. Factors associated with an increased chance of survival among patients suffering from an out-of-hospital cardiac arrest in a national perspective in Sweden. Am Heart J. 2005 Jan;149(1):61–6.
27. JENNINGS RB, SOMMERS HM, SMYTH GA, FLACK HA, LINN H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol. 1960 Jul;70:68–78.
28. Prasad SS, Russell M, Nowakowska M, Williams A, Yauk C. Gene expression analysis to identify molecular correlates of pre- and post-conditioning derived neuroprotection. J Mol Neurosci. 2012 Jun;47(2):322–39.
29. Zhao Z-Q, Morris CD, Budde JM, Wang N-P, Muraki S, Sun H-Y, et al. Inhibition of myocardial apoptosis reduces infarct size and improves regional contractile dysfunction during reperfusion. Cardiovasc Res. 2003 Jul;59(1):132–42.
30. Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D, Hausenloy DJ, et al. Postconditioning and protection from reperfusion injury: where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res. 2010 Aug;87(3):406–23.
31. Sanada S, Komuro I, Kitakaze M. Pathophysiology of myocardial reperfusion injury: preconditioning, postconditioning, and translational aspects of protective measures. Am J Physiol Heart Circ Physiol. 2011 Nov;301(5):H1723-41.
32. Tsang A, Hausenloy DJ, Yellon DM. Myocardial postconditioning: reperfusion injury revisited. Vol. 289, American journal of physiology. Heart and circulatory physiology. United States; 2005. p. H2-7.
33. Miura T, Tanno M, Sato T. Mitochondrial kinase signalling pathways in myocardial protection from ischaemia/reperfusion-induced necrosis. Cardiovasc Res. 2010 Oct;88(1):7–15.
34. Hausenloy DJ, Duchen MR, Yellon DM. Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. Cardiovasc Res. 2003 Dec;60(3):617–25.
35. Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. N Engl J Med. 2008 Jul;359(5):473–81.
36. Hausenloy DJ, Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway. Cardiovasc Res. 2004 Feb;61(3):448–60.
37. Schulman D, Latchman DS, Yellon DM. Urocortin protects the heart from reperfusion injury via upregulation of p42/p44 MAPK signaling pathway. Am J Physiol Heart Circ Physiol. 2002 Oct;283(4):H1481-8.
38. Yang X-M, Proctor JB, Cui L, Krieg T, Downey JM, Cohen M V. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell

signaling pathways. J Am Coll Cardiol. 2004 Sep;44(5):1103–10.
39. Hausenloy DJ, Yellon DM. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. Heart Fail Rev. 2007 Dec;12(3–4):217–34.
40. Hausenloy DJ, Yellon DM. Cardioprotective growth factors. Cardiovasc Res. 2009 Jul;83(2):179–94.
41. Dong S, Teng Z, Lu F-H, Zhao Y-J, Li H, Ren H, et al. Post-conditioning protects cardiomyocytes from apoptosis via PKC(epsilon)-interacting with calcium-sensing receptors to inhibit endo(sarco)plasmic reticulum-mitochondria crosstalk. Mol Cell Biochem. 2010 Aug;341(1–2):195–206.
42. Gao X, Zhang H, Takahashi T, Hsieh J, Liao J, Steinberg GK, et al. The Akt signaling pathway contributes to postconditioning's protection against stroke; the protection is associated with the MAPK and PKC pathways. J Neurochem. 2008 May;105(3):943–55.
43. Tong H, Chen W, Steenbergen C, Murphy E. Ischemic preconditioning activates phosphatidylinositol-3-kinase upstream of protein kinase C. Circ Res. 2000 Aug;87(4):309–15.
44. Juhaszova M, Zorov DB, Kim S-H, Pepe S, Fu Q, Fishbein KW, et al. Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J Clin Invest. 2004 Jun;113(11):1535–49.
45. Li H, Wei C, Gao J, Bai S, Li H, Zhao Y, et al. Mediation of dopamine D2 receptors activation in post-conditioning-attenuated cardiomyocyte apoptosis. Exp Cell Res. 2014 Apr;323(1):118–30.
46. Chai Y-S, Hu J, Lei F, Wang Y-G, Yuan Z-Y, Lu X, et al. Effect of berberine on cell cycle arrest and cell survival during cerebral ischemia and reperfusion and correlations with p53/cyclin D1 and PI3K/Akt. Eur J Pharmacol. 2013 May;708(1–3):44–55.
47. Kunuthur SP, Mocanu MM, Hemmings BA, Hausenloy DJ, Yellon DM. The Akt1 isoform is an essential mediator of ischaemic preconditioning. J Cell Mol Med. 2012 Aug;16(8):1739–49.
48. Tsang A, Hausenloy DJ, Mocanu MM, Yellon DM. Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. Circ Res. 2004 Aug;95(3):230–2.
49. Abdallah Y, Gkatzoflia A, Gligorievski D, Kasseckert S, Euler G, Schlu K, et al. Insulin protects cardiomyocytes against reoxygenation-induced hypercontracture by a survival pathway targeting SR Ca 2 + storage B. 2006;70:346–53.
50. Seger R, Krebs EG. The MAPK signaling cascade. FASEB J Off Publ Fed Am Soc Exp Biol. 1995 Jun;9(9):726–35.
51. Hussain A, Gharanei AM, Nagra AS, Maddock HL. Caspase inhibition via A3 adenosine receptors: a new cardioprotective mechanism against myocardial infarction. Cardiovasc drugs Ther. 2014 Feb;28(1):19–32.
52. Kim GS, Cho S, Nelson JW, Zipfel GJ, Han BH. TrkB agonist antibody pretreatment enhances neuronal survival and long-term sensory motor function following hypoxic ischemic injury in neonatal rats. PLoS One. 2014;9(2):e88962.

53. Lecour S. Activation of the protective Survivor Activating Factor Enhancement (SAFE) pathway against reperfusion injury: Does it go beyond the RISK pathway? J Mol Cell Cardiol. 2009 Jul;47(1):32–40.
54. Lacerda L, Somers S, Opie LH, Lecour S. Ischaemic postconditioning protects against reperfusion injury via the SAFE pathway. Cardiovasc Res. 2009 Nov;84(2):201–8.
55. Funamoto M, Fujio Y, Kunisada K, Negoro S, Tone E, Osugi T, et al. Signal transducer and activator of transcription 3 is required for glycoprotein 130-mediated induction of vascular endothelial growth factor in cardiac myocytes. J Biol Chem. 2000 Apr;275(14):10561–6.
56. Haghikia A, Ricke-Hoch M, Stapel B, Gorst I, Hilfiker-Kleiner D. STAT3, a key regulator of cell-to-cell communication in the heart. Cardiovasc Res. 2014 May;102(2):281–9.
57. Huffman LC, Koch SE, Butler KL. Coronary effluent from a preconditioned heart activates the JAK-STAT pathway and induces cardioprotection in a donor heart. Am J Physiol Heart Circ Physiol. 2008 Jan;294(1):H257-62.
58. Lecour S, Suleman N, Deuchar GA, Somers S, Lacerda L, Huisamen B, et al. Pharmacological preconditioning with tumor necrosis factor-alpha activates signal transducer and activator of transcription-3 at reperfusion without involving classic prosurvival kinases (Akt and extracellular signal-regulated kinase). Circulation. 2005 Dec;112(25):3911–8.
59. Gross ER, Hsu AK, Gross GJ. The JAK/STAT pathway is essential for opioid-induced cardioprotection: JAK2 as a mediator of STAT3, Akt, and GSK-3 beta. Am J Physiol Heart Circ Physiol. 2006 Aug;291(2):H827-34.
60. Wegrzyn J, Potla R, Chwae Y-J, Sepuri NB V, Zhang Q, Koeck T, et al. Function of mitochondrial Stat3 in cellular respiration. Science. 2009 Feb;323(5915):793–7.
61. Goodman MD, Koch SE, Fuller-Bicer GA, Butler KL. Regulating RISK: a role for JAK-STAT signaling in postconditioning? Am J Physiol Heart Circ Physiol. 2008 Oct;295(4):H1649-56.
62. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986 Nov;74(5):1124–36.
63. Reimer KA, Murry CE, Yamasawa I, Hill ML, Jennings RB. Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. Am J Physiol. 1986 Dec;251(6 Pt 2):H1306-15.
64. Sato H, Jordan JE, Zhao ZQ, Sarvotham SS, Vinten-Johansen J. Gradual reperfusion reduces infarct size and endothelial injury but augments neutrophil accumulation. Ann Thorac Surg. 1997 Oct;64(4):1099–107.
65. Okamoto F, Allen BS, Buckberg GD, Bugyi H, Leaf J. Reperfusion conditions: importance of ensuring gentle versus sudden reperfusion during relief of coronary occlusion. J Thorac Cardiovasc Surg. 1986 Sep;92(3 Pt 2):613–20.
66. Thuny F, Lairez O, Roubille F, Mewton N, Rioufol G, Sportouch C, et al. Post-conditioning reduces infarct size and edema in patients with ST-segment elevation myocardial infarction. J Am Coll Cardiol. 2012 Jun;59(24):2175–81.
67. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, et al. Postconditioning

the human heart. Circulation. 2005 Oct;112(14):2143–8.
68. Xie R, Wang P, Ji X, Zhao H. Ischemic post-conditioning facilitates brain recovery after stroke by promoting Akt/mTOR activity in nude rats. J Neurochem. 2013 Dec;127(5):723–32.
69. Wang Y, Ge P, Yang L, Wu C, Zha H, Luo T, et al. Protection of ischemic post conditioning against transient focal ischemia-induced brain damage is associated with inhibition of neuroinflammation via modulation of TLR2 and TLR4 pathways. J Neuroinflammation. 2014 Jan;11:15.
70. Ren C, Gao X, Niu G, Yan Z, Chen X, Zhao H. Delayed postconditioning protects against focal ischemic brain injury in rats. PLoS One. 2008;3(12):e3851.
71. Liu K-X, Li Y-S, Huang W-Q, Li C, Liu J, Li Y. Immediate but not delayed postconditioning during reperfusion attenuates acute lung injury induced by intestinal ischemia/reperfusion in rats: comparison with ischemic preconditioning. J Surg Res. 2009 Nov;157(1):e55-62.
72. Liu K-X, Li Y-S, Huang W-Q, Chen S-Q, Wang Z-X, Liu J-X, et al. Immediate postconditioning during reperfusion attenuates intestinal injury. Intensive Care Med. 2009 May;35(5):933–42.
73. Deftereos S, Giannopoulos G, Tzalamouras V, Raisakis K, Kossyvakis C, Kaoukis A, et al. Renoprotective effect of remote ischemic post-conditioning by intermittent balloon inflations in patients undergoing percutaneous coronary intervention. J Am Coll Cardiol. 2013 May;61(19):1949–55.
74. Segal N, Matsuura T, Caldwell E, Sarraf M, McKnite S, Zviman M, et al. Ischemic postconditioning at the initiation of cardiopulmonary resuscitation facilitates functional cardiac and cerebral recovery after prolonged untreated ventricular fibrillation. Resuscitation. 2012;83(11):1397–403.
75. Yannopoulos D, Segal N, Matsuura T, Sarraf M, Thorsgard M, Caldwell E, et al. Ischemic post-conditioning and vasodilator therapy during standard cardiopulmonary resuscitation to reduce cardiac and brain injury after prolonged untreated ventricular fibrillation. Resuscitation. 2013;84(8):1143–9.
76. Yannopoulos D, Segal N, McKnite S, Aufderheide TP, Lurie KG. Controlled pauses at the initiation of sodium nitroprusside-enhanced cardiopulmonary resuscitation facilitate neurological and cardiac recovery after 15 mins of untreated ventricular fibrillation. Crit Care Med. 2012 May;40(5):1562–9.
77. Inserte J, Barba I, Hernando V, Garcia-Dorado D. Delayed recovery of intracellular acidosis during reperfusion prevents calpain activation and determines protection in postconditioned myocardium. Cardiovasc Res. 2009 Jan;81(1):116–22.
78. Smith MA, Schnellmann RG. Calpains, mitochondria, and apoptosis. Cardiovasc Res. 2012 Oct;96(1):32–7.
79. Gao J, Guo J, Li H, Bai S, Li H, Wu B, et al. Involvement of dopamine D2 receptors activation in ischemic post-conditioning-induced cardioprotection through promoting PKC-epsilon particulate translocation in isolated rat hearts. Mol Cell Biochem. 2013 Jul;379(1–2):267–76.
80. Gulati P, Singh N, Muthuraman A. Pharmacologic evidence for role of endothelial

nitric oxide synthase in neuroprotective mechanism of ischemic postconditioning in mice. J Surg Res. 2014 May;188(1):349–60.
81. Kim J-S, Ohshima S, Pediaditakis P, Lemasters JJ. Nitric oxide: a signaling molecule against mitochondrial permeability transition- and pH-dependent cell death after reperfusion. Free Radic Biol Med. 2004 Dec;37(12):1943–50.
82. Kim J-S, Ohshima S, Pediaditakis P, Lemasters JJ. Nitric oxide protects rat hepatocytes against reperfusion injury mediated by the mitochondrial permeability transition. Hepatology. 2004 Jun;39(6):1533–43.
83. Schultz J, Segal N, Kolbeck J, McKnite S, Caldwell E, Yannopoulos D. Sodium nitroprusside enhanced cardiopulmonary resuscitation (SNPeCPR) improves vital organ perfusion pressures and carotid blood flow in a porcine model of cardiac arrest. Resuscitation. 2012 Mar;83(3):374–7.
84. Schultz J, Segal N, Kolbeck J, Caldwell E, Thorsgard M, McKnite S, et al. Sodium nitroprusside enhanced cardiopulmonary resuscitation prevents post-resuscitation left ventricular dysfunction and improves 24-hour survival and neurological function in a porcine model of prolonged untreated ventricular fibrillation. Resuscitation. 2011 Dec;82 Suppl 2:S35-40.
85. Schultz JC, Segal N, Caldwell E, Kolbeck J, McKnite S, Lebedoff N, et al. Sodium nitroprusside-enhanced cardiopulmonary resuscitation improves resuscitation rates after prolonged untreated cardiac arrest in two porcine models. Crit Care Med. 2011 Dec;39(12):2705–10.
86. Dezfulian C, Shiva S, Alekseyenko A, Pendyal A, Beiser DG, Munasinghe JP, et al. Nitrite therapy after cardiac arrest reduces reactive oxygen species generation, improves cardiac and neurological function, and enhances survival via reversible inhibition of mitochondrial complex I. Circulation. 2009 Sep;120(10):897–905.
87. Dezfulian C, Alekseyenko A, Dave KR, Raval AP, Do R, Kim F, et al. Nitrite therapy is neuroprotective and safe in cardiac arrest survivors. Nitric oxide Biol Chem. 2012 May;26(4):241–50.
88. Morrison RR, Tan XL, Ledent C, Mustafa SJ, Hofmann PA. Targeted deletion of A2A adenosine receptors attenuates the protective effects of myocardial postconditioning. Am J Physiol Heart Circ Physiol. 2007 Oct;293(4):H2523-9.
89. Philipp S, Yang X-M, Cui L, Davis AM, Downey JM, Cohen M V. Postconditioning protects rabbit hearts through a protein kinase C-adenosine A2b receptor cascade. Cardiovasc Res. 2006 May;70(2):308–14.
90. Olafsson B, Forman MB, Puett DW, Pou A, Cates CU, Friesinger GC, et al. Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: importance of the endothelium and the no-reflow phenomenon. Circulation. 1987 Nov;76(5):1135–45.
91. Babbitt DG, Virmani R, Forman MB. Intracoronary adenosine administered after reperfusion limits vascular injury after prolonged ischemia in the canine model. Circulation. 1989 Nov;80(5):1388–99.
92. Babbitt DG, Virmani R, Vildibill HDJ, Norton ED, Forman MB. Intracoronary adenosine administration during reperfusion following 3 hours of ischemia: effects on infarct size, ventricular function, and regional myocardial blood flow. Am

Heart J. 1990 Oct;120(4):808–18.
93. Kloner RA, Forman MB, Gibbons RJ, Ross AM, Alexander RW, Stone GW. Impact of time to therapy and reperfusion modality on the efficacy of adenosine in acute myocardial infarction: the AMISTAD-2 trial. Eur Heart J. 2006 Oct;27(20):2400–5.
94. Ross AM, Gibbons RJ, Stone GW, Kloner RA, Alexander RW. A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). J Am Coll Cardiol. 2005 Jun;45(11):1775–80.
95. Gill RS, Lee T-F, Manouchehri N, Liu J-Q, Lopaschuk G, Bigam DL, et al. Postresuscitation cyclosporine treatment attenuates myocardial and cardiac mitochondrial injury in newborn piglets with asphyxia-reoxygenation. Crit Care Med. 2013 Apr;41(4):1069–74.
96. Gill RS, Lee T-F, Sergi C, Bigam DL, Cheung P-Y. Early versus delayed cyclosporine treatment in cardiac recovery and intestinal injury during resuscitation of asphyxiated newborn piglets. Intensive Care Med. 2012 Jul;38(7):1215–23.
97. Gill RS, Manouchehri N, Liu J-Q, Lee T-F, Cho WJ, Thiesen A, et al. Cyclosporine treatment improves cardiac function and systemic hemodynamics during resuscitation in a newborn piglet model of asphyxia: a dose-response study. Crit Care Med. 2012 Apr;40(4):1237–44.
98. Huang C-H, Tsai M-S, Hsu C-Y, Su Y-J, Wang T-D, Chang W-T, et al. Post-cardiac arrest myocardial dysfunction is improved with cyclosporine treatment at onset of resuscitation but not in the reperfusion phase. Resuscitation. 2011 Dec;82 Suppl 2:S41-7.
99. Mewton N, Croisille P, Gahide G, Rioufol G, Bonnefoy E, Sanchez I, et al. Effect of cyclosporine on left ventricular remodeling after reperfused myocardial infarction. J Am Coll Cardiol. 2010 Mar;55(12):1200–5.
100. Wong GTC, Li R, Jiang LL, Irwin MG. Remifentanil post-conditioning attenuates cardiac ischemia-reperfusion injury via kappa or delta opioid receptor activation. Acta Anaesthesiol Scand. 2010 Apr;54(4):510–8.
101. Schultz JE, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by delta1- but not mu- or kappa-opioid receptors. Circulation. 1998 Apr;97(13):1282–9.
102. Schultz JJ, Hsu AK, Gross GJ. Ischemic preconditioning and morphine-induced cardioprotection involve the delta (delta)-opioid receptor in the intact rat heart. J Mol Cell Cardiol. 1997 Aug;29(8):2187–95.
103. Gross ER, Hsu AK, Gross GJ. Opioid-induced cardioprotection occurs via glycogen synthase kinase beta inhibition during reperfusion in intact rat hearts. Circ Res. 2004 Apr;94(7):960–6.
104. Ling Ling J, Wong GTC, Yao L, Xia Z, Irwin MG. Remote pharmacological post-conditioning by intrathecal morphine: cardiac protection from spinal opioid receptor activation. Acta Anaesthesiol Scand. 2010 Oct;54(9):1097–104.
105. Huhn R, Heinen A, Weber NC, Schlack W, Preckel B, Hollmann MW. Ischaemic

and morphine-induced post-conditioning: impact of mK(Ca) channels. Br J Anaesth. 2010 Nov;105(5):589–95.
106. Wu Q, Shen T, Ma H, Wang J. Sufentanil postconditioning protects the myocardium from ischemia-reperfusion via PI3K/Akt-GSK-3beta pathway. J Surg Res. 2012 Dec;178(2):563–70.
107. Hanouz JL, Vivien B, Gueugniaud PY, Lecarpentier Y, Coriat P, Riou B. Comparison of the effects of sevoflurane, isoflurane and halothane on rat myocardium. Br J Anaesth. 1998 May;80(5):621–7.
108. Chiari PC, Bienengraeber MW, Pagel PS, Krolikowski JG, Kersten JR, Warltier DC. Isoflurane protects against myocardial infarction during early reperfusion by activation of phosphatidylinositol-3-kinase signal transduction: evidence for anesthetic-induced postconditioning in rabbits. Anesthesiology. 2005 Jan;102(1):102–9.
109. Li L, Zuo Z. Isoflurane postconditioning induces neuroprotection via Akt activation and attenuation of increased mitochondrial membrane permeability. Neuroscience. 2011 Dec;199:44–50.
110. Li D, Huang B, Liu J, Li L, Li X. Decreased brain K(ATP) channel contributes to exacerbating ischemic brain injury and the failure of neuroprotection by sevoflurane post-conditioning in diabetic rats. PLoS One. 2013;8(8):e73334.
111. Ye R, Yang Q, Kong X, Li N, Zhang Y, Han J, et al. Sevoflurane preconditioning improves mitochondrial function and long-term neurologic sequelae after transient cerebral ischemia: role of mitochondrial permeability transition. Crit Care Med. 2012 Sep;40(9):2685–93.
112. Meybohm P, Gruenewald M, Albrecht M, Muller C, Zitta K, Foesel N, et al. Pharmacological postconditioning with sevoflurane after cardiopulmonary resuscitation reduces myocardial dysfunction. Crit Care. 2011;15(5):R241.
113. Fries M, Nolte KW, Coburn M, Rex S, Timper A, Kottmann K, et al. Xenon reduces neurohistopathological damage and improves the early neurological deficit after cardiac arrest in pigs. Crit Care Med. 2008 Aug;36(8):2420–6.
114. Ayoub IM, Kolarova J, Gazmuri RJ. Cariporide given during resuscitation promotes return of electrically stable and mechanically competent cardiac activity. Resuscitation. 2010 Jan;81(1):106–10.
115. Ayoub IM, Kolarova J, Kantola RL, Sanders R, Gazmuri RJ. Cariporide minimizes adverse myocardial effects of epinephrine during resuscitation from ventricular fibrillation. Crit Care Med. 2005 Nov;33(11):2599–605.
116. Lin X, Lee D, Wu D. Protective effects of NHE1 inhibition with sabiporide in an experimental model of asphyxia-induced cardiac arrest in piglets. Resuscitation. 2013 Apr;84(4):520–5.
117. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev. 2007 Dec;59(4):418–58.
118. Nichol G, Aufderheide TP, Eigel B, Neumar RW, Lurie KG, Bufalino VJ, et al. Regional systems of care for out-of-hospital cardiac arrest: A policy statement from the American Heart Association. Circulation. 2010 Feb;121(5):709–29.

119. Cummins RO. From concept to standard-of-care? Review of the clinical experience with automated external defibrillators. Ann Emerg Med. 1989 Dec;18(12):1269–75.
120. Hogler S, Sterz F, Sipos W, Schratter A, Weihs W, Holzer M, et al. Distribution of neuropathological lesions in pig brains after different durations of cardiac arrest. Resuscitation. 2010 Nov;81(11):1577–83.
121. Janata A, Bayegan K, Sterz F, Weihs W, Holzer M, Sipos W, et al. Limits of conventional therapies after prolonged normovolemic cardiac arrest in swine. Resuscitation. 2008 Oct;79(1):133–8.
122. Janata A, Weihs W, Schratter A, Bayegan K, Holzer M, Frossard M, et al. Cold aortic flush and chest compressions enable good neurologic outcome after 15 mins of ventricular fibrillation in cardiac arrest in pigs. Crit Care Med. 2010 Aug;38(8):1637–43.
123. Kern KB, Hilwig RW, Berg RA, Rhee KH, Sanders AB, Otto CW, et al. Postresuscitation left ventricular systolic and diastolic dysfunction. Treatment with dobutamine. Circulation. 1997 Jun;95(12):2610–3.
124. Garcia S, Henry TD, Wang YL, Chavez IJ, Pedersen WR, Lesser JR, et al. Long-term follow-up of patients undergoing postconditioning during ST-elevation myocardial infarction. J Cardiovasc Transl Res. 2011 Feb;4(1):92–8.
125. Yannopoulos D, Matsuura T, McKnite S, Goodman N, Idris A, Tang W, et al. No assisted ventilation cardiopulmonary resuscitation and 24-hour neurological outcomes in a porcine model of cardiac arrest. Crit Care Med. 2010;38(1):254–60.
126. Shultz JJ, Coffeen P, Sweeney M, Detloff B, Kehler C, Pineda E, et al. Evaluation of standard and active compression-decompression CPR in an acute human model of ventricular fibrillation. Circulation. 1994 Feb;89(2):684–93.
127. Marino BS, Yannopoulos D, Sigurdsson G, Lai L, Cho C, Redington A, et al. Spontaneous breathing through an inspiratory impedance threshold device augments cardiac index and stroke volume index in a pediatric porcine model of hemorrhagic hypovolemia. Crit Care Med. 2004 Sep;32(9 Suppl):S398-405.
128. Quinones MA, Waggoner AD, Reduto LA, Nelson JG, Young JB, Winters WLJ, et al. A new, simplified and accurate method for determining ejection fraction with two-dimensional echocardiography. Circulation. 1981 Oct;64(4):744–53.
129. Yu T, Weil MH, Tang W, Sun S, Klouche K, Povoas H, et al. Adverse outcomes of interrupted precordial compression during automated defibrillation. Circulation. 2002 Jul;106(3):368–72.
130. Berg RA, Sanders AB, Kern KB, Hilwig RW, Heidenreich JW, Porter ME, et al. Adverse hemodynamic effects of interrupting chest compressions for rescue breathing during cardiopulmonary resuscitation for ventricular fibrillation cardiac arrest. Circulation. 2001 Nov;104(20):2465–70.
131. Christenson J, Andrusiek D, Everson-Stewart S, Kudenchuk P, Hostler D, Powell J, et al. Chest compression fraction determines survival in patients with out-of-hospital ventricular fibrillation. Circulation. 2009 Sep;120(13):1241–7.
132. Cour M, Loufouat J, Paillard M, Augeul L, Goudable J, Ovize M, et al. Inhibition of mitochondrial permeability transition to prevent the post-cardiac arrest

syndrome: a pre-clinical study. Eur Heart J. 2011 Jan;32(2):226–35.
133. Sayre MR, Koster RW, Botha M, Cave DM, Cudnik MT, Handley AJ, et al. Part 5: Adult basic life support: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Circulation. 2010 Oct;122(16 Suppl 2):S298-324.
134. Shinohara G, Morita K, Nagahori R, Koh Y, Kinouchi K, Abe T, et al. Ischemic postconditioning promotes left ventricular functional recovery after cardioplegic arrest in an in vivo piglet model of global ischemia reperfusion injury on cardiopulmonary bypass. J Thorac Cardiovasc Surg. 2011 Oct;142(4):926–32.
135. Albrecht M, Gruenewald M, Zitta K, Zacharowski K, Scholz J, Bein B, et al. Hypothermia and anesthetic postconditioning influence the expression and activity of small intestinal proteins possibly involved in ischemia/reperfusion-mediated events following cardiopulmonary resuscitation. Resuscitation. 2012 Jan;83(1):113–8.
136. Shaffner DH, Eleff SM, Brambrink AM, Sugimoto H, Izuta M, Koehler RC, et al. Effect of arrest time and cerebral perfusion pressure during cardiopulmonary resuscitation on cerebral blood flow, metabolism, adenosine triphosphate recovery, and pH in dogs. Crit Care Med. 1999 Jul;27(7):1335–42.
137. Ayoub IM, Radhakrishnan J, Gazmuri RJ. Targeting mitochondria for resuscitation from cardiac arrest. Crit Care Med. 2008 Nov;36(11 Suppl):S440-6.
138. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection. Cardiovasc Res. 2004 Feb;61(3):372–85.
139. Weiss JN, Korge P, Honda HM, Ping P. Role of the mitochondrial permeability transition in myocardial disease. Circ Res. 2003 Aug;93(4):292–301.
140. Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, Ovize M. Postconditioning inhibits mitochondrial permeability transition. Circulation. 2005 Jan;111(2):194–7.
141. Argaud L, Gateau-Roesch O, Muntean D, Chalabreysse L, Loufouat J, Robert D, et al. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. J Mol Cell Cardiol. 2005 Feb;38(2):367–74.
142. Zhao H. Ischemic postconditioning as a novel avenue to protect against brain injury after stroke. J Cereb Blood Flow Metab [Internet]. 2009 May 25;29(5):873–85. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2736291/
143. Cour M, Gomez L, Mewton N, Ovize M, Argaud L. Postconditioning: from the bench to bedside. J Cardiovasc Pharmacol Ther. 2011 Jun;16(2):117–30.
144. Zeng Z, Huang HF, Chen MQ, Song F, Zhang YJ. Postconditioning prevents ischemia/reperfusion injury in rat liver transplantation. Hepatogastroenterology. 2010;57(101):875–81.
145. Snoeijs MGJ, van Heurn LWE, Buurman WA. Biological modulation of renal ischemia-reperfusion injury. Curr Opin Organ Transplant. 2010 Apr;15(2):190–9.
146. Gurusamy KS, Gonzalez HD, Davidson BR. Current protective strategies in liver surgery. World J Gastroenterol. 2010 Dec;16(48):6098–103.
147. Dreixler JC, Shaikh AR, Alexander M, Savoie B, Roth S. Post-ischemic

conditioning in the rat retina is dependent upon ischemia duration and is not additive with ischemic pre-conditioning. Exp Eye Res. 2010 Dec;91(6):844–52.
148. Ferencz A, Takacs I, Horvath S, Ferencz S, Javor S, Fekecs T, et al. Examination of protective effect of ischemic postconditioning after small bowel autotransplantation. Transplant Proc. 2010;42(6):2287–9.
149. Zhao W, Che X, Fan L, Wang S, Wang G, Zhang R, et al. [Protective effect of ischemic preconditioning against cold ischemia and reperfusion injury of rat small intestinal graft]. Nan Fang Yi Ke Da Xue Xue Bao. 2007 Nov;27(11):1764–6.
150. Lauzier B, Sicard P, Bouchot O, Delemasure S, Menetrier F, Moreau D, et al. After four hours of cold ischemia and cardioplegic protocol, the heart can still be rescued with postconditioning. Transplantation. 2007 Dec;84(11):1474–82.
151. Nichol G, Soar J. Regional cardiac resuscitation systems of care. Curr Opin Crit Care. 2010 Jun;16(3):223–30.
152. Nichol G, Thomas E, Callaway CW, Hedges J, Powell JL, Aufderheide TP, et al. Regional variation in out-of-hospital cardiac arrest incidence and outcome. JAMA. 2008 Sep;300(12):1423–31.
153. Grubb NR. Managing out-of-hospital cardiac arrest survivors: 2. Cardiological perspective. Vol. 85, Heart (British Cardiac Society). England; 2001. p. 123–4.
154. Grubb NR. Managing out-of-hospital cardiac arrest survivors: 1. Neurological perspective. Vol. 85, Heart (British Cardiac Society). England; 2001. p. 6–8.
155. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010 Jun;8(6):e1000412.
156. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. N Engl J Med. 2002 Feb;346(8):557–63.
157. Allen BS, Ko Y, Buckberg GD, Tan Z. Studies of isolated global brain ischaemia: III. Influence of pulsatile flow during cerebral perfusion and its link to consistent full neurological recovery with controlled reperfusion following 30 min of global brain ischaemia. Eur J Cardiothorac Surg. 2012 May;41(5):1155–63.
158. Allen BS, Ko Y, Buckberg GD, Tan Z. Studies of isolated global brain ischaemia: II. Controlled reperfusion provides complete neurologic recovery following 30 min of warm ischaemia - the importance of perfusion pressure. Eur J Cardiothorac Surg. 2012 May;41(5):1147–54.
159. Kin H, Zatta AJ, Lofye MT, Amerson BS, Halkos ME, Kerendi F, et al. Postconditioning reduces infarct size via adenosine receptor activation by endogenous adenosine. Cardiovasc Res. 2005 Jul;67(1):124–33.
160. Mykytenko J, Reeves JG, Kin H, Wang N-P, Zatta AJ, Jiang R, et al. Persistent beneficial effect of postconditioning against infarct size: role of mitochondrial K(ATP) channels during reperfusion. Basic Res Cardiol. 2008 Sep;103(5):472–84.
161. Back T, Schuler OG. The natural course of lesion development in brain ischemia. Acta Neurochir Suppl. 2004;89:55–61.
162. Shultz JJ, Mianulli MJ, Gisch TM, Coffeen PR, Haidet GC, Lurie KG. Comparison of exertion required to perform standard and active compression-

decompression cardiopulmonary resuscitation. Resuscitation. 1995 Feb;29(1):23–31.
163. Hausenloy DJ, Yellon DM. The therapeutic potential of ischemic conditioning: an update. Nat Rev Cardiol. 2011 Nov;8(11):619–29.
164. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? J Clin Invest. 1985 Nov;76(5):1713–9.
165. Knapp J, Bergmann G, Bruckner T, Russ N, Bottiger BW, Popp E. Pre- and postconditioning effect of Sevoflurane on myocardial dysfunction after cardiopulmonary resuscitation in rats. Resuscitation. 2013 Oct;84(10):1450–5.
166. Maskarinec SA, Hannig J, Lee RC, Lee KYC. Direct observation of poloxamer 188 insertion into lipid monolayers. Biophys J. 2002 Mar;82(3):1453–9.
167. Allen BS, Ko Y, Buckberg GD, Sakhai S, Tan Z. Studies of isolated global brain ischaemia: I. A new large animal model of global brain ischaemia and its baseline perfusion studies. Eur J Cardiothorac Surg. 2012 May;41(5):1138–46.
168. Yannopoulos D, McKnite S, Aufderheide TP, Sigurdsson G, Pirrallo RG, Benditt D, et al. Effects of incomplete chest wall decompression during cardiopulmonary resuscitation on coronary and cerebral perfusion pressures in a porcine model of cardiac arrest. Resuscitation. 2005 Mar;64(3):363–72.
169. Ristagno G, Tang W, Huang L, Fymat A, Chang Y-T, Sun S, et al. Epinephrine reduces cerebral perfusion during cardiopulmonary resuscitation. Crit Care Med. 2009 Apr;37(4):1408–15.
170. Ditchey R V, Lindenfeld J. Failure of epinephrine to improve the balance between myocardial oxygen supply and demand during closed-chest resuscitation in dogs. Circulation. 1988 Aug;78(2):382–9.
171. Tovar OH, Jones JL. Epinephrine facilitates cardiac fibrillation by shortening action potential refractoriness. J Mol Cell Cardiol. 1997 May;29(5):1447–55.
172. Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Harter L, Keel M, et al. Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. Anesthesiology. 2007 Feb;106(2):262–8.
173. Yasuda S, Townsend D, Michele DE, Favre EG, Day SM, Metzger JM. Dystrophic heart failure blocked by membrane sealant poloxamer. Nature. 2005 Aug;436(7053):1025–9.
174. Townsend D, Turner I, Yasuda S, Martindale J, Davis J, Shillingford M, et al. Chronic administration of membrane sealant prevents severe cardiac injury and ventricular dilatation in dystrophic dogs. J Clin Invest. 2010 Apr;120(4):1140–50.
175. Gu J-H, Ge J-B, Li M, Xu H-D, Wu F, Qin Z-H. Poloxamer 188 protects neurons against ischemia/reperfusion injury through preserving integrity of cell membranes and blood brain barrier. PLoS One. 2013;8(4):e61641.
176. Murphy AD, McCormack MC, Bichara DA, Nguyen JT, Randolph MA, Watkins MT, et al. Poloxamer 188 protects against ischemia-reperfusion injury in a murine hind-limb model. Plast Reconstr Surg. 2010 Jun;125(6):1651–60.
177. Walters TJ, Mase VJJ, Roe JL, Dubick MA, Christy RJ. Poloxamer-188 reduces muscular edema after tourniquet-induced ischemia-reperfusion injury in rats. J

Trauma. 2011 May;70(5):1192–7.
178. Bao H-J, Wang T, Zhang M-Y, Liu R, Dai D-K, Wang Y-Q, et al. Poloxamer-188 attenuates TBI-induced blood-brain barrier damage leading to decreased brain edema and reduced cellular death. Neurochem Res. 2012 Dec;37(12):2856–67.
179. Schaer GL, Spaccavento LJ, Browne KF, Krueger KA, Krichbaum D, Phelan JM, et al. Beneficial effects of RheothRx injection in patients receiving thrombolytic therapy for acute myocardial infarction. Results of a randomized, double-blind, placebo-controlled trial. Circulation. 1996 Aug;94(3):298–307.
180. Effects of RheothRx on mortality, morbidity, left ventricular function, and infarct size in patients with acute myocardial infarction. Collaborative Organization for RheothRx Evaluation (CORE). Circulation. 1997 Jul;96(1):192–201.
181. Daya MR, Schmicker RH, Morrison LJ. Current burden of cardiac arrest in the United States: report from the resuscitation outcomes consortium. 2015;
182. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. Nat Rev Cardiol. 2016 Apr;13(4):193–209.
183. Bartos JA, Matsuura TR, Sarraf M, Youngquist ST, McKnite SH, Rees JN, et al. Bundled postconditioning therapies improve hemodynamics and neurologic recovery after 17 min of untreated cardiac arrest. Resuscitation [Internet]. 2015;87:7–13. Available from: http://dx.doi.org/10.1016/j.resuscitation.2014.10.019
184. Heusch G. Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. Circ Res. 2015 Feb;116(4):674–99.
185. Council NR. Guide for the Care and Use of Laboratory Animals: Eighth Edition [Internet]. Washington, DC: The National Academies Press; 2011. Available from: https://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth
186. Riess ML, Matsuura TR, Bartos JA, Bienengraeber M, Aldakkak M, McKnite SH, et al. Anaesthetic Postconditioning at the Initiation of CPR Improves Myocardial and Mitochondrial Function in a Pig Model of Prolonged Untreated Ventricular Fibrillation. Resuscitation. 2014;85(12):1745–51.
187. Kristian T. Isolation of mitochondria from the CNS. Curr Protoc Neurosci. 2010 Jul;Chapter 7:Unit 7.22.
188. Camara AKS, Lesnefsky EJ, Stowe DF. Potential Therapeutic Benefits of Strategies Directed to Mitochondria. Antioxid Redox Signal [Internet]. 2010 Aug 1;13(3):279–347. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2936955/
189. Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature [Internet]. 2014 Nov 20;515(7527):431–5. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4255242/
190. Gazmuri RJ, Radhakrishnan J. Protecting mitochondrial bioenergetic function during resuscitation from cardiac arrest. Crit Care Clin. 2012 Apr;28(2):245–70.
191. Neumar RW, Brown CG, Van Ligten P, Hoekstra J, Altschuld RA, Baker P. Estimation of myocardial ischemic injury during ventricular fibrillation with total

circulatory arrest using high-energy phosphates and lactate as metabolic markers. Ann Emerg Med. 1991 Mar;20(3):222–9.
192. Aldakkak M, Stowe DF, Lesnefsky EJ, Heisner JS, Chen Q, Camara AKS. Modulation of mitochondrial bioenergetics in the isolated Guinea pig beating heart by potassium and lidocaine cardioplegia: implications for cardioprotection. J Cardiovasc Pharmacol. 2009 Oct;54(4):298–309.
193. Hurst S, Hoek J, Sheu S-S. Mitochondrial Ca2+ and regulation of the permeability transition pore. J Bioenerg Biomembr. 2016 Aug;
194. Lim SY, Davidson SM, Hausenloy DJ, Yellon DM. Preconditioning and postconditioning: the essential role of the mitochondrial permeability transition pore. Cardiovasc Res. 2007 Aug;75(3):530–5.
195. Marcu R, Neeley CK, Karamanlidis G, Hawkins BJ. Multi-parameter measurement of the permeability transition pore opening in isolated mouse heart mitochondria. J Vis Exp. 2012;(67).
196. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu S-S. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am J Physiol Cell Physiol. 2004 Oct;287(4):C817-33.
197. Tajeddine N. How do reactive oxygen species and calcium trigger mitochondrial membrane permeabilisation? Biochim Biophys Acta. 2016 Jun;1860(6):1079–88.
198. Gadicherla AK, Stowe DF, Antholine WE, Yang M, Camara AKS. Damage to mitochondrial complex I during cardiac ischemia reperfusion injury is reduced indirectly by anti-anginal drug ranolazine. Biochim Biophys Acta. 2012 Mar;1817(3):419–29.
199. Angelos MG, Kutala VK, Torres CA, He G, Stoner JD, Mohammad M, et al. Hypoxic reperfusion of the ischemic heart and oxygen radical generation. Am J Physiol Heart Circ Physiol. 2006 Jan;290(1):H341-7.
200. Touboul C, Angoulvant D, Mewton N, Ivanes F, Muntean D, Prunier F, et al. Ischaemic postconditioning reduces infarct size: systematic review and meta-analysis of randomized controlled trials. Arch Cardiovasc Dis. 2015 Jan;108(1):39–49.
201. Sharma V, Bell RM, Yellon DM. Targeting reperfusion injury in acute myocardial infarction: a review of reperfusion injury pharmacotherapy. Expert Opin Pharmacother. 2012 Jun;13(8):1153–75.
202. Coute RA, Mader TJ, Sherman LD. Outcomes by rescue shock number during the metabolic phase of porcine ventricular fibrillation resuscitation. Am J Emerg Med. 2014 Jun;32(6):586–91.
203. Yeh ST, Lee H-L, Aune SE, Chen C-L, Chen Y-R, Angelos MG. Preservation of mitochondrial function with cardiopulmonary resuscitation in prolonged cardiac arrest in rats. J Mol Cell Cardiol. 2009 Dec;47(6):789–97.
204. Bevers MB, Neumar RW. Mechanistic role of calpains in postischemic neurodegeneration. J Cereb Blood Flow Metab. 2008 Apr;28(4):655–73.
205. Kristian T. Metabolic stages, mitochondria and calcium in hypoxic/ischemic brain damage. Cell Calcium. 2004;36(3–4):221–33.
206. Lai TW, Zhang S, Wang YT. Excitotoxicity and stroke: identifying novel targets

for neuroprotection. Prog Neurobiol. 2014 Apr;115:157–88.
207. Xing B, Chen H, Zhang M, Zhao D, Jiang R, Liu X, et al. Ischemic postconditioning inhibits apoptosis after focal cerebral ischemia/reperfusion injury in the rat. Stroke. 2008 Aug;39(8):2362–9.
208. Kilbaugh TJ, Sutton RM, Karlsson M, Hansson MJ, Naim MY, Morgan RW, et al. Persistently Altered Brain Mitochondrial Bioenergetics After Apparently Successful Resuscitation From Cardiac Arrest. J Am Hear Assoc Cardiovasc Cerebrovasc Dis [Internet]. 2015 Sep 14;4(9):e002232. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4599507/
209. Reynolds JC, Salcido DD, Menegazzi JJ. Coronary Perfusion Pressure and Return of Spontaneous Circulation after Prolonged Cardiac Arrest. Prehosp Emerg Care [Internet]. 2010;14(1):78–84. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2922866/
210. Schwartz BG, Kloner RA. Coronary no reflow. J Mol Cell Cardiol. 2012 Apr;52(4):873–82.
211. Buchholz B, Donato M, D'Annunzio V, Gelpi RJ. Ischemic postconditioning: mechanisms, comorbidities, and clinical application. Mol Cell Biochem. 2014;392(1–2):1–12.
212. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976 May;72:248–54.
213. Myers CR, Antholine WE, Myers JM. The pro-oxidant chromium(VI) inhibits mitochondrial complex I, complex II, and aconitase in the bronchial epithelium: EPR markers for Fe-S proteins. Free Radic Biol Med. 2010 Dec;49(12):1903–15.
214. Weisfeldt ML, Becker LB. Resuscitation after cardiac arrest: a 3-phase time-sensitive model. JAMA. 2002 Dec;288(23):3035–8.
215. Matsuura TR, Bartos JA, Tsangaris A, Shekar KC, Olson MD, Riess ML, et al. Early Effects of Prolonged Cardiac Arrest and Ischemic Postconditioning during Cardiopulmonary Resuscitation on Cardiac and Brain Mitochondrial Function in Pigs. Resuscitation [Internet]. 2017; Available from: http://linkinghub.elsevier.com/retrieve/pii/S030095721730134X
216. Matsuura T, Bartos J, Sarraf M, Houang E, McKnite SH, Rees J, et al. Poloxamer 188 Improves Intra-CPR Hemodynamics and Post-Resuscitation LV Function in a Pig Model of Prolonged Cardiac Arrest. Circulation. 2013;128(22 Supplement):A282.
217. Dezfulian C, Kenny E, Lamade A, Misse A, Krehel N, St. Croix C, et al. Mechanistic characterization of nitrite-mediated neuroprotection after experimental cardiac arrest. J Neurochem. 2016;139(3):419–31.
218. Yannopoulos D, Bartos JA, George SA, Sideris G, Voicu S, Oestreich B, et al. Sodium nitroprusside enhanced cardiopulmonary resuscitation improves short term survival in a porcine model of ischemic refractory ventricular fibrillation. Resuscitation. 2017 Jan;110:6–11.
219. Halperin HR, Lee K, Zviman M, Illindala U, Lardo A, Kolandaivelu A, et al. Outcomes from low versus high-flow cardiopulmonary resuscitation in a swine

model of cardiac arrest. Am J Emerg Med [Internet]. 2010 Feb;28(2):195–202. Available from: http://www.sciencedirect.com/science/article/pii/S0735675709005300
220. Debaty G, Metzger A, Rees J, McKnite S, Puertas L, Yannopoulos D, et al. Enhanced Perfusion During Advanced Life Support Improves Survival With Favorable Neurologic Function in a Porcine Model of Refractory Cardiac Arrest. Crit Care Med [Internet]. 2015;1–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25756411
221. Nolan JP, Neumar RW, Adrie C, Aibiki M, Berg RA, Bbttiger BW, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication: a scientific statement from the International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Coun. Int Emerg Nurs. 2010 Jan;18(1):8–28.
222. Gold B, Puertas L, Davis SP, Metzger A, Yannopoulos D, Oakes DA, et al. Awakening after cardiac arrest and post resuscitation hypothermia: are we pulling the plug too early? Resuscitation. 2014 Feb;85(2):211–4.
223. Shah AP, Youngquist ST, McClung CD, Thomas JL, Tzvetkova E, Hanif MA, et al. Endothelin-1 attenuates the hemodynamic response to exogenous epinephrine in a porcine ischemic ventricular fibrillation cardiac arrest model. J Interferon Cytokine Res. 2011 Sep;31(9):679–84.
224. Buchholz B, D Annunzio V, Giani JF, Siachoque N, Dominici FP, Turyn D, et al. Ischemic postconditioning reduces infarct size through the alpha1-adrenergic receptor pathway. J Cardiovasc Pharmacol. 2014 Jun;63(6):504–11.
225. Debaty G, Lurie K, Metzger A, Lick M, Bartos JA, Rees JN, et al. Reperfusion injury protection during Basic Life Support improves circulation and survival outcomes in a porcine model of prolonged cardiac arrest. Resuscitation. 2016 Aug;105:29–35.
226. Radhakrishnan J, Wang S, Ayoub IM, Kolarova JD, Levine RF, Gazmuri RJ. Circulating levels of cytochrome c after resuscitation from cardiac arrest: a marker of mitochondrial injury and predictor of survival. Am J Physiol Heart Circ Physiol. 2007 Feb;292(2):H767-75.
227. Donnino MW, Liu X, Andersen LW, Rittenberger JC, Abella BS, Gaieski DF, et al. Characterization of mitochondrial injury after cardiac arrest (COMICA). Resuscitation. 2017 Apr;113:56–62.
228. Fang X, Huang Z, Zhu J, Jiang L, Li H, Fu Y, et al. Ultrastructural evidence of mitochondrial abnormalities in postresuscitation myocardial dysfunction. Resuscitation. 2012 Mar;83(3):386–94.
229. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox Biol. 2015;6.
230. Tullio F, Angotti C, Perrelli M-G, Penna C, Pagliaro P. Redox balance and cardioprotection. Basic Res Cardiol. 2013 Nov;108(6):392.
231. Bartos JA, Matsuura TR, Tsangaris A, Olson M, McKnite SH, Rees JN, et al. Intracoronary Poloxamer 188 Prevents Reperfusion Injury in a Porcine Model of ST-Segment Elevation Myocardial Infarction. JACC Basic to Transl Sci. 2016

Jun;1(4):224–34.
232. Cherry BH, Nguyen AQ, Hollrah RA, Olivencia-Yurvati AH, Mallet RT. Modeling cardiac arrest and resuscitation in the domestic pig. World J Crit care Med [Internet]. 2015;4(1):1–12. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4326759&tool=pmcentrez&rendertype=abstract
233. McCafferty K, Forbes S, Thiemermann C, Yaqoob MM. The challenge of translating ischemic conditioning from animal models to humans: the role of comorbidities. Dis Model Mech. 2014;7(12).
234. Heusch G, Rassaf T. Time to Give Up on Cardioprotection?: A Critical Appraisal of Clinical Studies on Ischemic Pre-, Post-, and Remote Conditioning??? Circ Res. 2016;119(5):676–95.
235. Heusch G, Gersh BJ. ERICCA and RIPHeart: Two nails in the coffin for cardioprotection by remote ischemic conditioning? Probably not! Eur Heart J. 2016;37(2):200–2.
236. Ferdinandy P, Hausenloy DJ, Heusch G, Baxter GF, Schulz R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. Pharmacol Rev. 2014;66(4).
237. Yannopoulos D, Aufderheide TP, Abella BS, Duval S, Frascone RJ, Goodloe JM, et al. Quality of CPR: An important effect modifier in cardiac arrest clinical outcomes and intervention effectiveness trials. Resuscitation [Internet]. 2015;94:106–13. Available from: http://dx.doi.org/10.1016/j.resuscitation.2015.06.004