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Thrombolytic-Enhanced Extracorporeal Cardiopulmonary Resuscitation After Prolonged Cardiac Arrest

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Abstract

Objective—To investigate the effects of the combination of extracorporeal cardiopulmonary resuscitation (ECPR) and thrombolytic therapy on the recovery of vital organ function after prolonged cardiac arrest.

Design—Laboratory investigation

Setting—University Laboratory

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Subjects—Pigs

Interventions—Animals underwent 30-minute untreated ventricular fibrillation cardiac arrest followed by extracorporeal cardiopulmonary resuscitation (ECPR) for 6 hours. Animals were allocated into two experimental groups: t-ECPR, which received Streptokinase 1 MU and c-ECPR which did not receive Streptokinase. In both groups the resuscitation protocol included the following physiologic targets: mean arterial pressure (MAP) > 70 mmHg, Cerebral perfusion pressure (CerPP) > 50 mmHg, PaO₂ 150 ± 50 mmHg, PaCO₂ 40 ± 5 mmHg and core temperature 33 ± 1 °C. Defibrillation was attempted after 30 minutes of ECPR.

Measurements and Main Results—A cardiac resuscitability score was assessed on the basis of: success of defibrillation; return of spontaneous heart beat; weanability from ECPR; and left ventricular systolic function after weaning. The addition of thrombolytic to ECPR significantly improved cardiac resuscitability (3.7 ± 1.6 in t-ECPR vs 1.0 ± 1.5 in c-ECPR). Arterial lactate clearance was higher in t-ECPR than in c-ECPR (40 ± 15% VS 18 ± 21 %). At the end of the experiment, the intracranial pressure was significantly higher in c-ECPR than in t-ECPR. Recovery of brain electrical activity, as assessed by quantitative analysis of EEG signal, and ischemic neuronal injury on histopathologic examination did not differ between groups. Animals in t-ECPR group did not have increased bleeding complications, including intracerebral hemorrhages.

Conclusions—In a porcine model of prolonged cardiac arrest, thrombolytic-enhanced ECPR improved cardiac resuscitability and reduced brain edema, without increasing bleeding complications. However, early EEG recovery and ischemic neuronal injury were not improved.

Keywords

Extracorporeal support; Cardiopulmonary Resuscitation; Thrombolytics; Animal models; Porcine; Prolonged Cardiac Arrest

Introduction

Sudden cardiac arrest (CA) is responsible for 1 out of every 5 deaths in the United States [1]. Average survival rates are < 20% for in-hospital CA (IHCA) [2] and < 11% for out-of-hospital CA (OHCA) [3–5]. A major limiting factor is failure to achieve return of spontaneous circulation (ROSC), with rates of sustained ROSC around 50% for IHCA [2] and 30% for OHCA [6]. Extracorporeal cardiopulmonary resuscitation (ECPR) is emerging as a feasible resuscitative strategy for patients who fail conventional resuscitative efforts, with reported survival rates ranging from 4% to 33% in selected patients [7–10]. However, ECPR alone often fails to reverse myocardial failure and severe neurologic damage when the interval from arrest onset to initiation of ECPR is prolonged [7, 9, 11–14]. The main causes of death in non-survivors resuscitated with ECPR are hemodynamic instability, multiple organ failure and irreversible brain injury [11, 15]. Therefore, additional therapeutic strategies (mechanical CPR, intra-arrest cooling) aimed at improving recovery of vital organ function after prolonged ischemia are required if ECPR is to be widely adopted to extend the duration of CA after which favorable outcome can be achieved [16].

In addition to ischemic injury, endothelial damage, and intravascular coagulation may limit recovery of organ function after prolonged CA [17–23]. Intravascular coagulation during cardiac arrest was initially described by Crowell in 1955 [24]. Fischer et al demonstrated that intravenous thrombolytic therapy given during CPR reduces the brain no-reflow phenomenon in animals after prolonged cardiac arrest [25]. Recent studies of organ in vivo perfusion for donation after cardiac death (DCD) have demonstrated that addition of thrombolytic agents to the initial perfusate enhances the function of transplanted organs [26, 27]. Since in vivo perfusion for DCD is typically performed after prolonged durations of untreated CA, we hypothesized that a similar strategy would be effective in ECPR. We tested this hypothesis in a newly developed swine model of prolonged untreated CA followed by resuscitation using ECPR.

Methods

All animals were treated in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guideline for the Care of Use of Laboratory Animals” published by the NIH [28]. This study was approved by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan.

Animal preparation

Fifteen pigs (44 ± 5 kg body weight) were utilized for this experiment. Animals were sedated with 5 mg/kg tiletamine and zolazepam and 3 mg/kg xylazine given intramuscularly, and anesthesia was induced with inhaled isoflurane (4.0 %). Endotracheal intubation was performed and mechanical ventilation instituted (tidal volume 10 ml/kg, respiratory rate adjusted to maintain a PaCO_2 of 40 ± 5 torr (5 ± 1 kPa), FiO_2 adjusted to maintain PaO_2 of 150 ± 50 torr (20 ± 7 kPa), PEEP 5 cmH₂O). Anesthesia was maintained with inhaled isoflurane 1.0–3.0%. Core temperature was maintained at $37.5 \pm 0.5^\circ\text{C}$ in the pre-arrest period. An initial 1000 mL bolus of normal saline was given intravenously, followed by continuous infusion of 200–500 mL/h to replace fluid loss. The animals did not receive any heparin prior to CA.

Hemodynamic monitoring—Lead II electrocardiography was continuously recorded. The right supraclavicular neck region was dissected and a subclavian arterial line was placed to monitor arterial blood pressure (Marquette Electronics, Milwaukee, WI). A pulmonary artery catheter was inserted via the right cephalic vein. Core temperature, mixed-venous oxygen saturation (S_vO_2), and cardiac output (thermodilution technique) were monitored using a Vigilance monitor (Edwards LifeSciences LLC, Irvine, CA). A transvenous right ventricular pacing wire was placed for induction of ventricular fibrillation (VF). A Foley catheter was placed into the bladder for urine output quantification.

Blood gases, lactate concentration and activated coagulation time (ACT) were repeatedly measured in arterial blood samples (ABL 725, Radiometer, Copenhagen, Denmark; Hemochron Blood Coagulation System, International Technidyne Corp. Edison, NJ).

Brain monitoring and analysis—For carotid arterial blood sampling, a 5 Fr catheter was introduced into the right carotid artery through small arteriotomy with maintenance of

distal flow. For intracerebral monitoring, bilateral burr holes were drilled into the skull. A brain oxygen tension/temperature probe (Licor, Integra, Plainsboro, NJ) and an intracranial pressure (ICP) catheter (Codman & Shurtleff, Inc. Raynham, MA) were inserted. ICP, cerebral perfusion pressure (CerPP = MAP-ICP) and brain tissue oxygen partial pressure (PbrO₂) were continuously monitored.

Cortical EEG activity was continuously recorded using 8 corkscrew scalp electrodes in a swine montage [29], labeled analogously to a human 10–20 montage (Natus, San Carlos, CA). EEG was sampled at 256 Hz, then analyzed using Magic Marker software (Persyst Corporation, San Diego). The following values were quantified for all recorded EEG: amplitude-integrated EEG [30], alpha-delta ratio (2 min windows) [31], standard deviation of EEG amplitude (10 s windows), spectral power integrated over 0–4 Hz, 4–8 Hz, 8–13 Hz, and 14–32 Hz (2 s windows), and two commercial algorithms (Artifact Probability and Burst Suppression Ratio, Persyst). The sliding 2-minute mean and standard deviation of each value was calculated from baseline and through the entire procedure. Periods of high likelihood for artifact were redacted. Post-CA measurements were normalized to the baseline values. The EEG analysis compared the values of each measurement with baseline (standard 2 sample pooled t-test) and assessed for any temporal trends that might indicate subtle improvement (Spearman correlation).

Surgical exposure for ECPR vascular access—Prior to initiation of cardiac arrest, surgical cut-downs were performed on the groin to expose the femoral artery and vein and on the left-side supraclavicular region to expose the subclavian vein in preparation for ECPR cannulation.

Cardiac arrest and ECPR initiation

A schematic diagram of the experiment design is provided in Figure 1. After instrumentation was completed and baseline data collected, ventricular fibrillation (VF) was electrically induced by delivering a 9V DC current through the ventricular pacing wire. VF was confirmed by ECG and arterial pressure tracing. Anesthesia and mechanical ventilation were discontinued. Normothermic no-flow CA was maintained for 30 minutes with no CPR.

Figure 2 is a schematic diagram of the ECPR circuit. Vascular access for ECPR was performed during CA. The previously exposed femoral artery and vein were cannulated using 18–20 Fr venous cannula for blood drainage and a 14–16 Fr arterial cannula for blood reinfusion. In 13 animals an additional 16–18 Fr venous drainage cannula was placed in the left subclavian vein to optimize venous drainage. Cannulas were secured and flushed with heparinized saline while awaiting initiation of ECPR. The ECPR system included a roller peristaltic pump (M-Pump, MC3, Ann Arbor, MI), a commercially available membrane oxygenator, 3/8-inch tubing and connectors. The system was primed with normal saline 500 mL, sodium bicarbonate 100 mEq, sodium heparin 8000 IU, vasopressin 20 IU, mannitol 12.5 g and methylprednisolone 500 mg. Animals were allocated into two experimental groups: **t-ECPR**, which received Streptokinase 1.0 MU added to the pump prime and **c-ECPR** which did not receive Streptokinase. After 30 min of untreated CA, the ECPR circuit

was turned on to restore systemic circulation. Sodium heparin infusion was started immediately after initiation of ECPR and titrated to keep an ACT of 250–350 seconds.

Resuscitation protocol

Initial reperfusion with ECPR was performed using a post-conditioning perfusion strategy (PCs), which is comprised of 5 cycles of interrupted flow (40 seconds on and 20 seconds off for 5 minutes) as described by Segal et al [32]. After the first 5 minutes of intermittent flow, continuous cardiopulmonary support was established with an ECPR blood flow > 50 mL/kg/min. Sweep gas to the oxygenator consisted of a mixture of oxygen and air allowing titration of F_iO_2 . ECPR was adjusted to achieve pre-set physiologic targets: Mean arterial pressure (MAP) > 70 mmHg [33], CerPP > 50 mmHg [34]; PaO_2 150 ± 50 torr (20 ± 7 kPa) [35, 36], and temperature-corrected $PaCO_2$ 40 ± 5 torr (5 ± 1 kPa) [33] in the carotid artery. Active cooling was started using ice packs in order to induce therapeutic hypothermia with a target temperature between $33 \pm 1^\circ C$. Surface cooling was chosen rather than a heat exchanger in the ECPR circuit to simplify the ECPR system for these experiments. ECPR support and the physiologic targets were maintained for 5 hours in all the animals.

After 30 minutes of ECPR support, external electrical defibrillation was attempted utilizing up to 4 shocks at 150–200J with a biphasic defibrillator (Philips Medical System, Andover, MA). If electrical shock was unsuccessful and VF persisted, repeat defibrillation was attempted after 60 and 120 minutes of ECPR. If VF recurred after initial successful defibrillation, up to 3 defibrillation attempts (200 J) were performed for each episode of VF. Vasopressin infusion was used if needed to achieve target perfusion pressures. Dobutamine was used to for inotropic support during ECPR weaning. If return of spontaneous heart beat (ROSB) occurred, mechanical ventilation was resumed and FiO_2 and minute ventilation adjusted to maintain PaO_2 and $PaCO_2$ targets. After 5 hours of controlled perfusion, weaning from ECPR was attempted in animals with ROSB.

Six hours after initiation of ECPR, animals were euthanized by intravenous injection of pentobarbital sodium. Immediately upon euthanasia, brain was fixed by bilateral carotid perfusion with 1L of heparinized saline (10 UI/mL) followed by 1L of 4% formalin flush. Brain was post-fixed in 10% formalin until the time of processing for gross and histopathologic examination.

Outcome evaluation

Cardiovascular recovery—Cardiac resuscitability was assessed on the basis of: success of defibrillation; return of spontaneous heart beat (ROSB); weanability from ECPR; and left ventricular systolic function (LVSF) after weaning. These components were summarized into a “cardiac resuscitability score” (Table 1).

Successful defibrillation was defined as restoration of a stable supraventricular cardiac rhythm. ROSB was defined as sustained evidence of organized ventricular contractions based on transthoracic echocardiography (TTE). A TTE was performed prior to arrest, after return of stable supraventricular rhythm and then every hour during ECPR. In the animals with ROSB, weanability from ECPR was assessed during the last hour of the experiment.

ECPR blood flow was progressively decreased while titrating vasopressor support. Weaning was determined successful if a stable MAP > 65 mmHg could be maintained with no ECPR flow.

In the animals successfully weaned from ECPR, a final standardized TTE was recorded for a semiquantitative assessment of global left ventricular function (left ventricular shortening fraction, LVSF). Parasternal short axis views were obtained, video clips recorded, and files uploaded for review by two pediatric cardiologists, who were blinded to the treatment group. For each animal baseline (pre-arrest) and final (post-ECPR) clips were graded both qualitatively and quantitatively. Qualitative assessment included grading global LVSF as normal or mildly, moderately or severely diminished. Quantitative assessment included measuring % fractional shortening whenever degree of systolic function and standardized views permitted accurate assessment.

Arterial blood samples—Blood was collected from the carotid arterial line prior to CA, 10 minutes after ECPR initiation, 30 minutes after ECPR initiation and then every hour until the end of the experiment. Measured parameters included pH, PaO₂, PaCO₂, hemoglobin, arterial lactate, electrolytes, and ACT. Six-hour lactate clearance was calculated as: *six-hour lactate clearance = (peak lactate concentration – final lactate concentration) / peak lactate concentration * 100*

Cerebral recovery—Assessment of cerebral recovery was based on: changes in ICP and PbrO₂ after CA and during ECPR; return of brain electrical activity on EEG; quantification of brain hemorrhages and acute ischemic neurodegeneration. Continuously recorded EEG was reviewed by a board certified epileptologist, blinded to study group, to assess whether any significant brain activity had returned. Formalin-fixed brain was cut into 5 mm serial coronal sections and imaged for assessment of intracerebral hemorrhage. Total section area and hemorrhagic area were quantified using the Image Pro Premier software, and the relative area with hemorrhage calculated as percent of total area imaged. Brain was then processed for paraffin histology using standardized dissections of selectively vulnerable brain regions (dorsal hippocampus, cerebellar vermis, caudal putamen, parietal cortex, and thalamus) to ischemia. Acute neuronal degeneration was scored on 0–5 scale (0= 0% neuronal injury, 1=1% – 20%, 2=21% – 40%, 3=41% – 60%, 4=61% – 80%, 5=81% – 100%) by estimating the percentage of neurons showing ischemic cytopathology in each region.

Statistical analysis

All parameters were tested for normality using the Shapiro-Wilks test. Continuous variables with a normal distribution are expressed as mean ± standard deviation and compared using Student t-test. Continuous variables without a normal distribution are expressed as median and interquartile range and compared using Mann-Whitney U-test. For repeated measurements two-way analysis of variance was used to detect intergroup differences and corrected with the Bonferroni method for multiple comparison. Categorical variables are compared using Fisher exact test. P < 0.05 were considered significant.

Results

Out of 15 animals entered in the study, 2 animals were excluded from data analysis due to technical difficulties with venous access resulting in ECPR blood flow < 50 mL/kg/min. In 13 animals (n=7 in t-ECPR group; n=6 in c-ECPR group) ECPR was successfully initiated 30 minutes after VF induction. Baseline characteristics were similar between the two groups (Table 2).

Physiologic parameters of reperfusion—Pre-determined physiologic targets were achieved within the first 30 minutes of ECPR (Table 3) and maintained throughout the study in both groups (Figure 3, Panel A-D). Time to achieve target temperature for therapeutic hypothermia was 69 ± 23 min in t-ECPR and 70 ± 25 min in c-ECPR ($p = 0.95$). ACT was maintained in the therapeutic range in both groups (290 ± 55 seconds in t-ECPR and 310 ± 71 seconds in c-ECPR, $p = 0.59$), and the required dose of heparin did not differ between groups (1353 ± 304 U/h in t-ECPR and 1163 ± 221 U/h in c-ECPR, $p = 0.26$). Average vasopressin dose over the duration of the experiment was similar between groups (0.37 ± 0.20 U/min in t-ECPR and 0.4 ± 0.2 U/min in c-ECPR, $p = 0.67$).

Cardiovascular recovery—Cardiac resuscitability for the two groups is summarized in Figure 4. All the animals in t-ECPR (7 of 7) and 3 out of 6 animals in c-ECPR group could be defibrillated into stable supraventricular rhythm. Defibrillation success rate was not statistically different between the two groups ($p=0.07$). ROSB occurred in 7 of 7 (100%) animals in t-ECPR group and in 1 of 6 (17%) animals in c-ECPR group ($p < 0.01$). Four of 7 animals (57%) in t-ECPR group and 1 of 6 (17%) animals in c-ECPR were successfully weaned from ECPR ($p=0.27$). After weaning, LVSF was moderately diminished in the 4 animals in t-ECPR and severely diminished in the animal in c-ECPR group. Cardiac resuscitability score was 3.7 ± 1.6 in t-ECPR group and 1.0 ± 1.5 in c-ECPR group ($p < 0.05$) using Mann-Whitney U-test (Table 4).

Arterial blood samples and urine output—Arterial lactate concentration markedly increased after CA. t-ECPR group had significantly lower lactate concentration at the peak level (11.6 ± 0.7 vs 12.6 ± 0.6 , $p < 0.05$) at 3 and 4 hours (Figure 3G). Six-hour lactate clearance was significantly higher in t-ECPR than in c-ECPR (40 ± 15 % vs 18 ± 21 %, $p < 0.05$). Hemoglobin concentration at 6 hours was not significantly different between groups (8.0 ± 1.5 in t-ECPR and 8.5 ± 2.2 in c-ECPR, $p = 0.63$). During ECPR urine output was restored in both groups.

Cerebral recovery—After 5 hours of ECPR, before starting weaning attempts, ICP was significantly increased from baseline in the c-ECPR (34 ± 16 mmHg, $p < 0.01$), but not in the t-ECPR group (16 ± 3 ; $p = 0.37$). The absolute increase in ICP was 19 ± 13 mmHg in c-ECPR and 1 ± 3 mmHg in t-ECPR group ($p < 0.05$). Comparing ICP between the two groups, values were significantly higher in c-ECPR group than in t-ECPR at 6 hours (Figure 3E). As expected, PbrO₂ dropped during CA in both groups (0.7 ± 0.5 torr (0.1 ± 0.06 kPa) in t-ECPR, 0.9 ± 0.4 torr (0.12 ± 0.05) in c-ECPR, $p = 0.64$). ECPR initiation was followed by a rise in

PbrO₂ in both groups. No significant difference in PbrO₂ values between the groups was observed throughout the experiment (Figure 3F). Quantitative analysis of EEG signal revealed no recovery of brain activity in either group. Burst suppression ratio remained unchanged or worse, and the EEG power was unchanged in all spectral bands (Table 5). Both groups had some evidence of intracerebral hemorrhages at pathologic examination. The extent of hemorrhagic areas as a percentage of the entire brain was significantly smaller in c-ECPR than in t-ECPR ($1.1 \pm 0.7\%$ vs $0.2 \pm 0.2\%$, $p < 0.05$) (Figure 5 and supplemental Figure 1). The overall histopathologic neurodegeneration score was not different between groups (3.2 ± 1.1 in t-ECPR and 3.2 ± 0.9 in c-ECPR, $p = 0.97$). The regional neurodegeneration scores are presented in (Figure 6)

Discussion

This study tested the hypothesis that thrombolytic-enhanced ECPR improves resuscitation and organ function recovery after prolonged CA. Our results demonstrate that adding a thrombolytic agent to the ECPR initial perfusate improves cardiac resuscitability while reducing brain edema. These effects were observed without significant bleeding complications. However, we were unable to detect an early effect on EEG recovery or ischemic neuronal injury.

Intravascular coagulation after prolonged cardiac arrest—It has long been known that prolonged CA is associated with a marked activation of blood coagulation [18, 37, 38]. Crowell et al originally described intravascular coagulation during cardiac arrest in the 1950s [24]. In these early studies, Crowell also demonstrated that pretreatment with intravenous heparin resulted in a dose-dependent improvement in cardiac arrest survival [24]. More recently, White et al using whole thromboelastography (TEG) observed that clot initiation time (R) was shortened and clot strength (MA) tended to increase at 8 minutes of untreated VF [22]. One study suggests that a diffuse endothelial damage may occur after CA [19] and the subsequent exposition of tissue factor is likely to be an important contributing mechanism to the post-arrest prothrombotic state [21]. Budrham et al detected left ventricular thrombus by transesophageal echocardiography (TEE) in 86% of swine within 6 minutes of untreated ventricular fibrillation [17]. Finally, in humans treated with standard CPR, Varriale et al reported echocardiographic detection of intracardiac thrombus at 20–30 minutes after cardiac arrest onset, a finding that will be uniformly associated with failed resuscitation [23]. ECPR after out-of-hospital cardiac arrest is typically initiated 30–60 minutes after cardiac arrest onset, making the likelihood of intravascular coagulation high in these patients.

Intravascular coagulation could represent a limiting factor to restoring tissue perfusion after ischemia, creating a “no-reflow” phenomenon in which there is an absence of microvascular flow despite return of circulation [19, 39, 40]. The role of intravascular coagulation in the pathogenesis of reperfusion disturbances after CA is supported by the observations that pre-arrest administration of heparin or streptokinase improves survival and neurologic outcome [37, 38, 41]. Moreover, Fischer et al demonstrated that the no-reflow phenomenon in the

brain is reduced by thrombolytic administration during standard CPR after prolonged cardiac arrest [25]. The severity and extent of brain no-reflow increase with the duration of untreated CA [19]. The secondary injury caused by no-reflow appears to become relevant after durations of untreated CA longer than 12–15 minutes [42]. With ECPR return of circulation is achieved after periods of CA long enough to allow for intravascular coagulation causing the no-reflow phenomenon, and thus the potential for improving reperfusion could be maximized with thrombolytic agents. Streptokinase pre-flush during *ex situ* and *in situ* perfusion of kidneys from donors after cardiac death (DCD) has been shown to improve function of transplanted organs after up to 60 minutes of asystole [26, 27]. Organs transplanted after *in situ* perfusion during DCD procurement undergo the same pathophysiological events of ischemia-reperfusion as organs resuscitated after prolonged CA including the possible occurrence of no-reflow phenomenon. Therefore it is conceivable that, similar to its effects in DCD, thrombolytic therapy can improve functional organ recovery after prolonged CA.

Optimizing initial reperfusion with ECPR—The quality of early reperfusion affects organ function recovery after prolonged ischemia [34, 35, 43, 44]. Effective control of reperfusion conditions (including MAP, PaO₂, PaCO₂ and temperature) is one of the mechanisms proposed to explain the superiority of ECPR over CPR [43, 45]. In developing this model we adopted the current state of literature in terms of optimization of initial reperfusion after CA. The levels of MAP that we maintained (70 – 90 mmHg) are in line with the recommendations of the International Liaison Committee on Resuscitation (ILCOR) [33]. Allen et al, reported that a CerPP of 50 mmHg is needed to minimize brain injury after 30 minutes of global brain ischemia in swine [34]. Vasopressin was chosen as the vasopressor agent because experimental studies in pigs have demonstrated beneficial effects in the improvement of vital organ blood flow [46] and cerebral microcirculation [47] after CA. The target for PaO₂ (150±50 torr, (20±7 kPa)) was selected to provide adequate oxygen delivery, while limiting the potential detrimental effects of hyperoxia during early reperfusion [35, 36]. Of note, strict control of PaO₂ becomes more challenging after ROSB, because repeated adjustments of both ECPR and ventilator settings are required when native cardiac output is restored. This difficulty explains the slight difference in PaO₂ between the two groups at 3 and 4 hours. PaCO₂ was maintained within the normal range in order to avoid secondary ischemia caused by cerebral vasoconstriction and increased ICP caused by cerebral vasodilation. Mild therapeutic hypothermia was applied according to current guidelines [33], with target temperature of 33±1°C attained within 2 hours of initiating ECPR.

Cardiovascular Recovery with t-ECPR—Our results indicate that thrombolytic-enhanced ECPR increases cardiac resuscitability. During VF the effectiveness of defibrillation diminishes over time. Optimization of myocardial perfusion is required to restore myocardial conditions favorable for successful defibrillation [48]. The rate of successful defibrillation in the c-ECPR group (50%) corresponds to what reported in an early dog study with the same duration of CA followed by ECPR [14]. Early decline of heart function after resuscitation is associated with a parallel impairment of myocardial microcirculation [49]. In line with our findings, an earlier study by Fisher et al has

demonstrated that thrombolytics improve recovery of cardiac contractility after prolonged (15 minute) CA and standard CPR [25]. Better lactate clearance also indicates improved reperfusion with the use of thrombolytics. The higher lactate concentrations and the lower lactate clearance in the c-ECPR group suggest a greater persistence of anaerobic metabolism and thus inadequate organ perfusion without the use of thrombolytics.

Cerebral Recovery with t-ECPR—In our study thrombolytic-enhanced ECPR prevents the progressive rise in ICP which is likely to occur during the early reperfusion after prolonged CA. ICP changes after CA have not been widely investigated. Safar reported no significant rise in ICP after 15 minutes of global ischemia in dogs [50], but marked cerebral edema develops after 30 minutes of global brain ischemia followed by uncontrolled reperfusion in pigs [51]. The progressive rise in ICP with c-ECPR is therefore likely caused by persistent or progressive multifocal brain ischemia, resulting in increasing cerebral edema.

Considering the relatively short duration of observation, the absence of recovery of brain electrical activity on EEG in either group is not unexpected, and does not exclude the potential for recovery of function with longer post-arrest observation times [52].

Surprisingly, the rise in ICP in the c-ECPR group was not associated with more severe ischemic neurodegeneration at the histologic examination. Since cytotoxic brain edema represents the initial step in the progression of ischemic neuronal injury, it is conceivable that the histopathologic changes were still developing at the time of brain harvesting. Indeed, delayed onset and progression of neurodegeneration up to 72 hours after the ischemic insult have been reported in experimental models of transient global cerebral ischemia [53]. An alternative hypothesis is that the beneficial effect of thrombolytic therapy on ICP was mediated through effects on non-neuronal cells including the brain endothelium and/or glial cells.

Improvement of early reperfusion is likely to be only one component of a complex strategy to prevent neuronal injury after prolonged CA. In experimental studies of isolated global brain ischemia, Allen et al [34] demonstrated that controlled reperfusion provides complete neurologic recovery after 30 minutes of warm ischemia. Nevertheless, models of isolated organ ischemia fail to fully reproduce the pathophysiology of CA: secondary brain injury after resuscitation may result from the consequences of whole-body ischemia, which include cardiac and respiratory dysfunction with impairment of systemic perfusion and oxygenation and systemic release of inflammatory mediators.

Complications of t-ECPR—Reported incidence of major hemorrhagic complications during ECPR ranges from 14% [8] to 29% [15]. Risk of bleeding is the major drawback of the use of thrombolytics after CA and it is potentially magnified by the systemic anticoagulation required for ECPR. However, thrombolytic therapy was not associated with increased bleeding complications in this study. Conversely, the average extent of brain hemorrhages was significantly smaller in t-ECPR group. This seemingly paradoxical result might be explained by the effectiveness of thrombolytic therapy in reducing ischemic injury. Extensive brain infarctions have been reported in porcine models of 30-minute global brain

ischemia and reperfusion [34] and hemorrhagic conversion of ischemic infarctions is potentially facilitated by heparinization during ECPR. By decreasing the number or size of ischemic infarctions, thrombolytic therapy may result in a lower overall incidence of intracerebral hemorrhage. Importantly, both the choice of lytic agent and the mode of administration may affect the risk of hemorrhagic adverse effect. The *in-vivo* half-life of streptokinase is 30 minutes; therefore single bolus administration results in a relatively short-term activation of fibrinolysis, which may mitigate the risk of bleeding.

Study Limitations—The relatively short period of observation after CA limits the assessment of long-term recovery of organ function in this experimental study. A 30-minute period of untreated CA followed by ECPR is unlikely to occur in the clinical setting where standard CPR is likely to precede ECPR. The rationale for using this study design was to more efficiently test the hypothesis that thrombolytics improve functional recovery of vital organs after prolonged CA. Adding CPR to the model would have created significant variability, thus affecting the possibility to discern the impact of thrombolytic therapy. We acknowledge that different combinations of no-flow and low-flow time, together with the quality of CPR will have an impact on the underlying pathophysiology of intravascular coagulation and the effectiveness of treatments intended to improve early organ reperfusion.

Another limitation of our approach is that we did not quantify the extent of intravascular coagulation in the organs of the experimental animals. We instead elected to examine the clinically relevant outcomes of functional organ recovery. Therefore, we do not have a direct evidence to demonstrate that the differences in outcome between the two groups were caused by reversal of intravascular coagulation. Direct measurements of intravascular coagulation in future studies using more mechanistically oriented models are needed. These studies will be essential to characterize the time course and location of intravascular coagulation during prolonged cardiac arrest, identify the most effective and safe thrombolytic agents, and optimize the dose and duration of thrombolytic therapy.

Finally, the evaluation of cerebral resuscitation was based on physiologic surrogates (ICP and PbrO₂), EEG, and histology, with a relatively short recovery period of 6 hours. The relationship between these measurements and ultimate recovery of neurologic function remains to be determined in this experimental model. We do expect that a period of at least 7 days will be needed reliably assess ultimate recovery of neurologic function. Long-term outcome studies will be essential to demonstrate the impact of thrombolytic-enhanced ECPR on survival with good neurologic function.

Conclusion

The combination of ECPR and thrombolytic therapy after prolonged untreated CA improves recovery of cardiac function and reduces brain edema without increasing bleeding complications. However, we were unable to detect an effect on early EEG recovery or ischemic neuronal injury. Longer term outcome studies will be needed to fully elucidate the impact of thrombolytic-enhanced ECPR on cardiovascular and cerebral recovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Mozaffarian DBE, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2015 update: a report from the american heart association. *Circulation*. 2015; 131(4):e29–e322. [PubMed: 25520374]
2. Girotra S, Nallamothu BK, Spertus JA, Li Y, Krumholz HM, Chan PS. American Heart Association Get with the Guidelines-Resuscitation I. Trends in survival after in-hospital cardiac arrest. *The New England journal of medicine*. 2012; 367(20):1912–1920. [PubMed: 23150959]
3. Nichol G, Thomas E, Callaway CW, Hedges J, Powell JL, Aufderheide TP, Rea T, Lowe R, Brown T, Dreyer J, et al. Regional variation in out-of-hospital cardiac arrest incidence and outcome. *JAMA : the journal of the American Medical Association*. 2008; 300(12):1423–1431. [PubMed: 18812533]
4. Sasson C, Rogers MA, Dahl J, Kellermann AL. Predictors of survival from out-of-hospital cardiac arrest: a systematic review and meta-analysis. *Circulation Cardiovascular quality and outcomes*. 2010; 3(1):63–81. [PubMed: 20123673]
5. Cardiac Arrest Registry to Enhance Survival (CARES) National Summary Report. 2013. <https://mycaresnet/>
6. (CARES) CARES. 2013 CARES National Summary Report. 2013.
7. Chen YS, Yu HY, Huang SC, Lin JW, Chi NH, Wang CH, Wang SS, Lin FY, Ko WJ. Extracorporeal membrane oxygenation support can extend the duration of cardiopulmonary resuscitation. *Critical care medicine*. 2008; 36(9):2529–2535. [PubMed: 18679121]
8. Le Guen M, Nicolas-Robin A, Carreira S, Raux M, Leprince P, Riou B, Langeron O. Extracorporeal life support following out-of-hospital refractory cardiac arrest. *Critical care*. 2011; 15(1):R29. [PubMed: 21244674]
9. Maekawa K, Tanno K, Hase M, Mori K, Asai Y. Extracorporeal cardiopulmonary resuscitation for patients with out-of-hospital cardiac arrest of cardiac origin: a propensity-matched study and predictor analysis. *Critical care medicine*. 2013; 41(5):1186–1196. [PubMed: 23388518]
10. Sakamoto TMN, Nagao K, Asai Y, Yokota H, Nara S, Hase M, Tahara Y, Atsumi T. SAVE-J Study Group. Extracorporeal cardiopulmonary resuscitation versus conventional cardiopulmonary resuscitation in adults with out-of-hospital cardiac arrest: a prospective observational study. *Resuscitation*. 2014; 85(6):762–768. [PubMed: 24530251]
11. Kagawa E, Inoue I, Kawagoe T, Ishihara M, Shimatani Y, Kurisu S, Nakama Y, Dai K, Takayuki O, Ikenaga H, et al. Assessment of outcomes and differences between in- and out-of-hospital cardiac arrest patients treated with cardiopulmonary resuscitation using extracorporeal life support. *Resuscitation*. 2010; 81(8):968–973. [PubMed: 20627526]
12. Liakopoulos OJ, Allen BS, Buckberg GD, Hristov N, Tan Z, Villablanca JP, Trummer G. Resuscitation after prolonged cardiac arrest: role of cardiopulmonary bypass and systemic hyperkalemia. *The Annals of thoracic surgery*. 2010; 89(6):1972–1979. [PubMed: 20494058]
13. Kano T, Hashiguchi A, Sadanaga M, Ashimura K, Sakamoto M, Morioka T. Cardiopulmonary-cerebral resuscitation by using cardiopulmonary bypass through the femoral vein and artery in dogs. *Resuscitation*. 1993; 25(3):265–281. [PubMed: 8351424]

14. Reich H, Angelos M, Safar P, Sterz F, Leonov Y. Cardiac resuscitability with cardiopulmonary bypass after increasing ventricular fibrillation times in dogs. *Annals of emergency medicine*. 1990; 19(8):887–890. [PubMed: 2372171]
15. Fagnoul D, Taccone FS, Belhaj A, Rondelet B, Argacha JF, Vincent JL, Backer DD. Extracorporeal life support associated with hypothermia and normoxemia in refractory cardiac arrest. *Resuscitation*. 2013; 84(11):1519–1524. [PubMed: 23816899]
16. Stub D, Bernard S, Pellegrino V, Smith K, Walker T, Sheldrake J, Hockings L, Shaw J, Duffy SJ, Burrell A, et al. Refractory cardiac arrest treated with mechanical CPR, hypothermia, ECMO and early reperfusion (the CHEER trial). *Resuscitation*. 2014
17. Budhram GRMT, Lutfy L, Murman D, Almulhim. Left ventricular thrombus development during ventricular fibrillation and resolution during resuscitation in a swine model of sudden cardiac arrest. *Resuscitation*. 2014; 85:689–693. [PubMed: 24518559]
18. Bottiger BW, Motsch J, Bohrer H, Boker T, Aulmann M, Nawroth PP, Martin E. Activation of blood coagulation after cardiac arrest is not balanced adequately by activation of endogenous fibrinolysis. *Circulation*. 1995; 92(9):2572–2578. [PubMed: 7586359]
19. Fischer M, Hossmann KA. No-reflow after cardiac arrest. *Intensive care medicine*. 1995; 21(2): 132–141. [PubMed: 7775694]
20. Grundmann S, Fink K, Rabadzheva L, Bourgeois N, Schwab T, Moser M, Bode C, Busch HJ. Perturbation of the endothelial glycocalyx in post cardiac arrest syndrome. *Resuscitation*. 2012; 83(6):715–720. [PubMed: 22306259]
21. Callaway CW. Endothelial damage after cardiac arrest--"endotheliitis". *Resuscitation*. 2012; 83(6): 667–668. [PubMed: 22429972]
22. White NJLB, Brueckner J, Martin EJ, Brophy DF, Peberdy MA, Ornato J, Ward KR. Coagulopathy during cardiac arrest and resuscitation in a swine model of electrically induced ventricular fibrillation. *Resuscitation*. 2011; 82:925–931. [PubMed: 21482008]
23. Varriale PMJ. Echocardiographic observations during in hospital cardiopulmonary resuscitation. *Critical care medicine*. 1997; 25:1717–1720. [PubMed: 9377888]
24. Crowell JWSG, Lambright RL, Read WL. The mechanism of death after resuscitation following acute circulatory failure. *Surgery*. 1955; 38(4):696–702. [PubMed: 13256276]
25. Fischer M, Bottiger BW, Popov-Cenic S, Hossmann KA. Thrombolysis using plasminogen activator and heparin reduces cerebral no-reflow after resuscitation from cardiac arrest: an experimental study in the cat. *Intensive care medicine*. 1996; 22(11):1214–1223. [PubMed: 9120116]
26. Gok MA, Shenton BK, Buckley PE, Peaston R, Cornell C, Soomro N, Jaques BC, Manas DM, Talbot D. How to improve the quality of kidneys from non-heart-beating donors: a randomised controlled trial of thrombolysis in non-heart-beating donors. *Transplantation*. 2003; 76(12):1714–1719. [PubMed: 14688521]
27. Reznik ON, Skvortsov AE, Reznik AO, Ananyev AN, Tutin AP, Kuzmin DO, Bagnenko SF. Uncontrolled donors with controlled reperfusion after sixty minutes of asystole: a novel reliable resource for kidney transplantation. *PloS one*. 2013; 8(5):e64209. [PubMed: 23737973]
28. Guide for the Care and Use of Laboratory Animals. Washington DC: National Academy of Sciences; 1996.
29. Weenink RPVX, van Putten MJ, Hollmann MW, Stevens MF, van Gulik TM, van Hulst RA. Quantitative electroencephalography in a swine model of cerebral arterial gas embolism. *Clin Neurophysiol*. 2012; 123(2):411–417. [PubMed: 21802358]
30. Ito AMY, Koga Y, Saho M, Hiraki T, Ushijima K. The use of amplitude-integrated electroencephalography combined with continuous conventional electroencephalography during therapeutic hypothermia in an infant with postnatal cardiac arrest. *Springerplus*. 2014; 21(3)
31. Claassen JHL, Kreiter KT, Du EY, Connolly ES, Emerson RG, Mayer SA. Quantitative continuous EEG for detecting delayed cerebral ischemia in patients with poor-grade subarachnoid hemorrhage. *Clin Neurophysiol*. 2004; 115(12):2699–2710. [PubMed: 15546778]
32. Segal N, Matsuura T, Caldwell E, Sarraf M, McKnite S, Zviman M, Aufderheide TP, Halperin HR, Lurie KG, Yannopoulos D. Ischemic postconditioning at the initiation of cardiopulmonary

resuscitation facilitates functional cardiac and cerebral recovery after prolonged untreated ventricular fibrillation. *Resuscitation*. 2012; 83(11):1397–1403. [PubMed: 22521449]

33. Neumar RW, Nolan JP, Adrie C, Aibiki M, Berg RA, Bottiger BW, Callaway C, Clark RS, Geocadin RG, Jauch EC, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A consensus statement from the International Liaison Committee on Resuscitation (American Heart Association, Australian and New Zealand Council on Resuscitation, European Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Asia, and the Resuscitation Council of Southern Africa); the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; and the Stroke Council. *Circulation*. 2008; 118(23):2452–2483. [PubMed: 18948368]
34. 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. *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery*. 2012; 41(5):1147–1154. [PubMed: 22436245]
35. Vereczki V, Martin E, Rosenthal RE, Hof PR, Hoffman GE, Fiskum G. Normoxic resuscitation after cardiac arrest protects against hippocampal oxidative stress, metabolic dysfunction, and neuronal death. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2006; 26(6):821–835.
36. Kilgannon JH, Jones AE, Shapiro NI, Angelos MG, Milcarek B, Hunter K, Parrillo JE, Trzeciak S. Emergency Medicine Shock Research Network I. Association between arterial hyperoxia following resuscitation from cardiac arrest and in-hospital mortality. *JAMA : the journal of the American Medical Association*. 2010; 303(21):2165–2171. [PubMed: 20516417]
37. Crowell JW, Sharpe GP, Lambright RL, Read WL. The mechanism of death after resuscitation following acute circulatory failure. *Surgery*. 1955; 38(4):696–702. [PubMed: 13256276]
38. Crowell JWSE. Effect of fibrinolytic activation on survival and cerebral damage following periods of circulatory arrest. *The American journal of physiology*. 1956; 186(2):283–285. [PubMed: 13362523]
39. Darius H, Yanagisawa A, Brezinski ME, Hock CE, Lefer AM. Beneficial effects of tissue-type plasminogen activator in acute myocardial ischemia in cats. *Journal of the American College of Cardiology*. 1986; 8(1):125–131. [PubMed: 3086417]
40. Jerome SN, Akimitsu T, Korthuis RJ. Leukocyte adhesion, edema, and development of postischemic capillary no-reflow. *The American journal of physiology*. 1994; 267(4 Pt 2):H1329–1336. [PubMed: 7943378]
41. Gaszynski W. Use of protease inhibitor (trasyolol) and heparin in cardiorespiratory resuscitation. II. Gasometric investigations of arterial blood. *Anaesth Resusc Intensive Ther*. 1975; 3(3):203–211. [PubMed: 3120]
42. Lin SR, O'Connor MJ, Fischer HW, King A. The effect of combined dextran and streptokinase on cerebral function and blood flow after cardiac arrest: and experimental study on the dog. *Invest Radiol*. 1978; 13(6):490–498. [PubMed: 755026]
43. Safar P, Xiao F, Radovsky A, Tanigawa K, Ebmeier U, Bircher N, Alexander H, Stezoski SW. Improved cerebral resuscitation from cardiac arrest in dogs with mild hypothermia plus blood flow promotion. *Stroke; a journal of cerebral circulation*. 1996; 27(1):105–113.
44. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *The New England journal of medicine*. 2007; 357(11):1121–1135. [PubMed: 17855673]
45. Trummer G, Foerster K, Buckberg GD, Benk C, Heilmann C, Mader I, Feuerhake F, Liakopoulos O, Brehm K, Beyersdorf F. Successful resuscitation after prolonged periods of cardiac arrest: a new field in cardiac surgery. *The Journal of thoracic and cardiovascular surgery*. 2010; 139(5):1325–1332. 1332 e1321–1322. [PubMed: 20412965]
46. Wenzel V, Lindner KH, Prengel AW, Maier C, Voelckel W, Lurie KG, Strohmenger HU. Vasopressin improves vital organ blood flow after prolonged cardiac arrest with postcountershock pulseless electrical activity in pigs. *Critical care medicine*. 1999; 27(3):486–492. [PubMed: 10199526]

47. Ristagno G, Sun S, Tang W, Castillo C, Weil MH. Effects of epinephrine and vasopressin on cerebral microcirculatory flows during and after cardiopulmonary resuscitation. *Critical care medicine*. 2007; 35(9):2145–2149. [PubMed: 17855828]
48. Kolarova J, Ayoub IM, Yi Z, Gazmuri RJ. Optimal timing for electrical defibrillation after prolonged untreated ventricular fibrillation. *Critical care medicine*. 2003; 31(7):2022–2028. [PubMed: 12847399]
49. Kern KB, Zuercher M, Cragun D, Ly S, Quash J, Bhartia S, Hilwig RW, Berg RA, Ewy GA. Myocardial microcirculatory dysfunction after prolonged ventricular fibrillation and resuscitation. *Critical care medicine*. 2008; 36(11 Suppl):S418–421. [PubMed: 20449904]
50. Snyder JV, Nemoto EM, Carroll RG, Safar P. Global ischemia in dogs: intracranial pressures, brain blood flow and metabolism. *Stroke; a journal of cerebral circulation*. 1975; 6(1):21–27.
51. Allen BS, Ko Y, Buckberg GD, Sakhal S, Tan Z. Studies of isolated global brain ischaemia: I. A new large animal model of global brain ischaemia and its baseline perfusion studies. *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery*. 2012; 41(5):1138–1146. [PubMed: 22436249]
52. Wijdicks EF, Hijdra A, Young GB, Bassetti CL, Wiebe S. Quality Standards Subcommittee of the American Academy of N. Practice parameter: prediction of outcome in comatose survivors after cardiopulmonary resuscitation (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*. 2006; 67(2):203–210. [PubMed: 16864809]
53. Pulsinelli WA, Brierley JB, Plum F. Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Annals of neurology*. 1982; 11(5):491–498. [PubMed: 7103425]

Figure 1. Schematic Diagram of ECPR Study Protocol

After 30 minutes of untreated VF cardiac arrest, animals in both the experimental groups undergo the same 6-hour resuscitation protocol which included: ECPR support with pre-set physiologic targets (“therapeutic targets”) for perfusion pressures, blood gases, temperature and anticoagulation; defibrillation attempts 30 minutes after ECPR start; ECPR weaning trial during the last hour of the experiment. Outcome evaluation focused on cardiovascular and cerebral recovery. Animals in t-ECPR group received Streptokinase 1.0 MU at ECPR initiation, while animals in c-ECPR group did not receive Streptokinase.

Figure 2. Schematic Diagram of Porcine ECPR Model

Anesthetized animals were intubated and connected to a mechanical ventilator. Animal instrumentation included: arterial line for continuous blood pressure monitoring; carotid and jugular sampling lines for blood collection; a brain oxygen tension/temperature probe (Licox probe) and an intracranial pressure catheter (ICP probe) for intracerebral monitoring; scalp electrodes to record cortical electroencephalographic activity. ECPR was performed using a veno-arterial (V-A) extracorporeal circuit, with blood drainage from femoral and subclavian vein and blood reinfusion into the femoral artery. The circuit included drainage and reinfusion cannulas, a roller peristaltic pump, an oxygenator, tubing and connectors.

Figure 3. Physiologic and Metabolic Parameters During ECPR

(A) Mean arterial pressure (MAP), (B) Cerebral perfusion pressure (CerPP), (C) Arterial PO₂ (PaO₂) from carotid artery, (D) Arterial PCO₂ (PaCO₂, panel D) from carotid artery, (E) Intracranial pressure (ICP), (F) Brain oxygen tension (PbrO₂), and (G) Arterial lactate concentration during ECPR. Data are presented as mean ± standard deviation. *p < 0.05 between groups (two-way ANOVA with Bonferroni correction)

Figure 4. Cardiac Resuscitability Outcomes

Defibrillation success was defined as return of stable supraventricular rhythm. Return of spontaneous heart beating (ROSB) was assessed on the basis of the evidence of heart contraction by qualitative echocardiography. Weaning from ECPR was determined successful if a stable MAP > 65 mmHg could be maintained with no ECPR flow. Semi-quantitative assessment of left ventricular function after weaning was performed by two blinded echocardiographers using standardized echocardiographic view. All the animals in t-ECPR group (7 of 7) could be successfully defibrillated into stable supraventricular rhythm. In the c-ECPR group, 3 of 6 animals (50%) achieved restoration of stable supraventricular rhythm; in 2 of them defibrillation was successful at 30 minutes after ECPR initiation, while 1 had persistent refractory VF and was successfully defibrillated after intravenous administration of amiodarone 150 mg. Of the remaining 3 animals in c-ECPR group, 1 had recurrent VF with supraventricular rhythm restored only transiently despite repeat defibrillation attempts and 2 had degeneration into asystole. See Table 4 for calculation of cardiac resuscitability score.

Figure 5. Brain Hemorrhage Quantification

Brain hemorrhages were measured on serial brain slices using Image-Pro Premier software, and expressed in percentage of the area imaged for the entire brain. The results presented as mean $\pm$ SD of percentage. Mann-Whitney unpaired t test was using to compare groups. Statistical significance was set at p value < 0.05 .

A. Representative Brain Histopathology

B. Regional Neurodegeneration Scores

Figure 6. Regional Histopathology and Neurodegeneration Scores

Neuronal damage in selectively vulnerable brain regions was quantified in 5 μ m sections stained with hematoxylin and eosin (H&E). Each region was divided into nine 40x fields using a standardized method. Within each field the region with the most neuron damage was imaged. For each animal, an average score of neurodegeneration from the nine fields was obtained for each region. Morphologic criteria for acute ischemic neurodegeneration included cytoplasmic eosinophilia, neuronal cell body shrinkage and angulation, and nuclear pyknosis.

(A). Brain histopathology of three representative animals from the studies was shown at five specific brain regions that stained by H&E and imaged at 40x Magnification. The acute ischemic degenerating neurons (pyknosis of nucleus, shrinkage and angulation cell body, and eosinophilia of cytoplasm) were seen at all regions in the two groups. Neurons in the sham animal represent normal histological appearance.

(B). Regional neurodegeneration scores. Quantification of neurodegeneration scores at five brain regions and overall score. The data expressed as a given score that based on the estimated percentage of degenerating neurons in each region. The results presented as mean

$\pm$ SD of the scores. One-Way ANOVA was used for analysis of variance, followed by Tukey's multiple comparisons test. $p < 0.05$ was considered statistically significant. There was no significant difference between the two groups at each individual region.

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TABLE 1

Cardiac resuscitability score

Components of Cardiac Resuscitability	Grades (= Score)			
	Yes (=1)		No (=0)	
Success of defibrillation	Yes (=1)		No (=0)	
ROSB	Yes (=1)		No (=0)	
Weanability from ECPR	Yes (=1)		No (=0)	
LVSF after weaning	Normal or mildly depressed (=3)	Moderately depressed (=2)	Severely depressed (=1)	Not weanable (=0)

Cardiac resuscitability score was calculated according to four components: success of defibrillation (0–1 points), ROSB (0–1 points), weanability from ECPR (0–1 points) and LVSF after weaning (0–3 points). The maximum score is 6: successfully defibrillated into stable supraventricular rhythm, with ROSB, weaned from ECPR, with normal or mildly depressed LVSF after weaning. The minimum score is 0: unsuccessful defibrillation.

Table 2

Baseline characteristics between groups

VARIABLE	c-ECPR (<i>n</i> = 6)	t-ECPR (<i>n</i> = 7)	p value
CO (ml/kg/min)	81 ± 19	100 ± 20	ns
MAP (mmHg)	94 ± 16	89 ± 14	ns
pH	7.46 ± 0.04	7.44 ± 0.05	ns
PaO ₂ (mmHg)	172 ± 15	159 ± 23	ns
PaCO ₂ (mmHg) #	40.1 (37.6–41)	39.7 (34.5–45.2)	ns
Hemoglobin (g/dL) #	11.9 (10.4–12.5)	9.7 (7.9–11.1)	ns
PbrO ₂ (mmHg)	20.1 ± 7.3	14.6 ± 7.2	ns
ICP (mmHg)	14.2 ± 5.4	14.4 ± 2.4	ns
CPP (mmHg)	80 ± 18	74 ± 16	ns
Lactate (mmol/L)	1.7 ± 0.5	1.1 ± 0.3	ns
Temp (°C)	37.4 ± 0.4	37.6 ± 0.3	ns

Baseline characteristics of the c-ECPR and t-ECPR groups.

non-normally distributed values presented as median (range). Statistical significance set at a p value <0.05.

Table 3

Goal-directed physiologic parameters

Variable	c-ECPR (<i>n</i> = 6)	t-ECPR (<i>n</i> = 7)	target
ECPR Blood flow (ml/kg/min)	54 ± 7	65 ± 11	>50
MAP (mmHg)	75 ± 14	76 ± 9	>70
CPP (mmHg)	56 (49–62)	58 (50–64)	>50
PaO ₂ (mmHg)	203 ± 66	161 ± 53	100–200
PaCO ₂ (mmHg) [#]	45.2 (39.8–54.8)	40.3 (34.5–45.2)	35–45

Goal-directed physiologic variables at 30 minutes after ECPR initiation.

Table 4

Cardiac resuscitability score

	c-ECPR group					t-ECPR group				
Animal #	Success of defib.	ROSB	Wean From ECPR	LV/SF after wean	Score	Success of defib.	ROSB	Wean From ECPR	LV/SF after wean	Score
1	1	0	0	0	1	1	1	1	2	5
2	0	0	0	0	0	1	1	1	2	5
3	0	0	0	0	0	1	1	0	0	2
4	1	0	0	0	1	1	1	1	2	5
5	0	0	0	0	0	1	1	0	0	2
6	1	1	1	1	4	1	1	1	2	5
7						1	1	0	0	2
Average score (mean $\pm$ standard deviation)					1 ± 1.5					

Table 5

Quantitative EEG analysis

	ADR	BSR	aEEG	Amp Std	0–4 Hz	4–8 Hz	9–13 Hz	14–32 Hz
T Stat	0.82	0.45	0.70	0.70	0.60	0.70	0.55	0.65
Spearman	0.04 *	0.45	0.74	0.74	0.60	0.50	0.60	0.60

Quantitative EEG analysis compared the effect of t-ECPR with c-ECPR. During CA, EEG signals changed drastically, which was quantified with several different measurements taken every 10 seconds. After ROSB, these measurements did not improve in either group. The only value trending toward significance was the Spearman correlation of Alpha/Delta Ratio (ADR), which as a two-tailed test needed p<0.025 to be significant. T Stat: raw statistic from the 2-sample t-test, comparing the distribution of each measurement during CA vs. after ROSB. Spearman: Spearman correlation of each measurement after ROSB vs. time. ADR: Alpha/Delta Ratio, BSR: Burst Suppression Ratio, aEEG: amplitude-integrated EEG, Amp Std: Amplitude standard deviation, last 4 columns: spectral band power of indicated frequency ranges.