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Therapeutic Effect of Argatroban During Cardiopulmonary Resuscitation and Streptokinase During Extracorporeal Cardiopulmonary Resuscitation in a Porcine Model of Prolonged Cardiac Arrest

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OBJECTIVE: Prolonged cardiac arrest (CA) causes microvascular thrombosis which is a potential barrier to organ reperfusion during extracorporeal cardiopulmonary resuscitation (ECPR). The aim of this study was to test the hypothesis that early intra-arrest anticoagulation during cardiopulmonary resuscitation (CPR) and thrombolytic therapy during ECPR improve recovery of brain and heart function in a porcine model of prolonged out-of-hospital CA.

DESIGN: Randomized interventional trial.

SETTING: University laboratory.

SUBJECTS: Swine.

INTERVENTIONS: In a blinded study, 48 swine were subjected to 8 minutes of ventricular fibrillation CA followed by 30 minutes of goal-directed CPR and 8 hours of ECPR. Animals were randomized into four groups (n = 12) and given either placebo (P) or argatroban (ARG; 350 mg/kg) at minute 12 of CA and either placebo (P) or streptokinase (STK, 1.5 MU) at the onset of ECPR.

MEASUREMENTS AND MAIN RESULTS: Primary outcomes included recovery of cardiac function measured by cardiac resuscitability score (CRS: range 0–6) and recovery of brain function measured by the recovery of somatosensory-evoked potential (SSEP) cortical response amplitude. There were no significant differences in recovery of cardiac function as measured by CRS between groups (p = 0.16): P + P 2.3 (1.0); ARG + P = 3.4 (2.1); P + STK = 1.6 (2.0); ARG + STK = 2.9 (2.1). There were no significant differences in the maximum recovery of SSEP cortical response relative to baseline between groups (p = 0.73): P + P = 23% (13%); ARG + P = 20% (13%); P + STK = 25% (14%); ARG + STK = 26% (13%). Histologic analysis demonstrated reduced myocardial necrosis and neurodegeneration in the ARG + STK group relative to the P + P group.

CONCLUSIONS: In this swine model of prolonged CA treated with ECPR, early intra-arrest anticoagulation during goal-directed CPR and thrombolytic therapy during ECPR did not improve initial recovery of heart and brain function but did reduce histologic evidence of ischemic injury. The impact of this therapeutic strategy on the long-term recovery of cardiovascular and neurological function requires further investigation.

KEY WORDS: animal models; anticoagulants; cardiopulmonary resuscitation; extracorporeal support; thrombolytics

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KEY POINTS

Question: Does early intra-arrest anticoagulation during cardiopulmonary resuscitation (CPR) and thrombolytic therapy during extracorporeal cardiopulmonary resuscitation (ECPR) improve recovery of brain and heart function in a porcine model of prolonged out-of-hospital cardiac arrest?

Findings: Outcomes included recovery of cardiac function measured by cardiac resuscitability score and recovery of brain function measured by the recovery of somatosensory-evoked potential cortical response amplitude. There were no significant differences in either primary outcome between groups.

Meaning: Early intra-arrest anticoagulation during CPR and thrombolytic therapy during ECPR did not improve initial recovery of heart and brain function, and therapeutic strategies for long-term recovery require further investigation.

Extracorporeal cardiopulmonary resuscitation (ECPR) is an effective resuscitation strategy for patients with cardiac arrest (CA) refractory to conventional cardiopulmonary resuscitation (CPR) (1–4). However, numerous patients treated with ECPR after prolonged CA die due to severe brain injury. Laboratory studies suggest that a fundamental barrier to successful ECPR after prolonged CA could be the "no-reflow phenomenon," which results in inadequate vital organ reperfusion despite restoring arterial blood flow and perfusion pressure (5, 6). Potential causes of the "no-reflow phenomenon" include microvascular thrombosis (6), leukocyte adhesion (7), and neutrophil extracellular trap formation (8). Clinical strategies to reduce no-reflow caused by microvascular thrombosis include anticoagulation during CPR and thrombolytic therapy at the initiation of ECPR. In this study, we test the hypothesis that the combination of early intra-arrest anticoagulation during CPR and thrombolytic therapy at the onset of ECPR would improve recovery of brain and heart function in a porcine model of prolonged CA. We chose ARG as the anticoagulant because it directly inhibits both circulating and clot-bound thrombin without the requirement of cofactors to exert its antithrombotic action (9, 10). We chose streptokinase (STK) for thrombolysis based on our previous study demonstrating that STK added to the ECPR prime circuit improved recovery of cardiac function in swine after prolonged CA (11).

MATERIALS AND METHODS

All animal procedures were performed at the University of Michigan Extracorporeal Life Support Laboratory. Animal husbandry was provided by staff of the Unit for Laboratory Animal Medicine (ULAM) under the guidance of supervisors who are certified as animal technologists by the American Association for Laboratory Animal Science. Veterinary care was provided by ULAM faculty members and veterinary residents. The animal care and use program conforms to the standards in "The Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies eighth edition (revised 2011). Protocol #00009358 was approved on February 11, 2020, by the University of Michigan Institutional Animal Care and Use Committee.

Induction and Anesthesia

Anesthesia was induced using intramuscular injection (5 mg/kg tiletamine and zolazepam and 3 mg/kg xylazine) followed by intubation and inhaled isoflurane (1–3%) with standard volume ventilation: tidal volume 7–9 mL/kg, respiratory rate adjusted to maintain a $Paco_2$ = of 40 ± 5 mm Hg, Fio_2 = 0.21–0.5 (adjusted to maintain Pao_2 = 100–200 mm Hg), PEEP = 5 cm H_2o.

Hemodynamic Monitoring

Intravascular catheters were placed in the right subclavian artery for mean arterial pressure (MAP) and subclavian vein for central venous pressure. A pulmonary artery catheter was placed in the subclavian vein for hemodynamic monitoring (Marquette Electronics, Milwaukee, WI). Core temperature, mixed-venous oxygen saturation, and cardiac output by thermodilution technique were monitored by a Vigilance Monitor (Edwards LifeSciences LLC, Irvine, CA). A Foley catheter was placed to monitor urine output. Before initiation of CA, surgical cut-downs and 4 Fr percutaneous sheath catheters (M00115710B1; Boston Scientific, Marlborough, MA) were placed in the groin to expose the right femoral artery and left femoral vein in preparation for ECPR

cannulation. A multichannel data acquisition system (MP150; Biopac, Goleta, CA) recorded all hemodynamic monitoring throughout the CA period. Standard echocardiographic views were acquired using a diagnostic ultrasound system (ACUSON Cypress, Siemens Medical Solutions USA, Mountain View, CA) following the technique described by Kerut et al (12).

Microcirculatory blood flow measurements of the sublingual mucosa were obtained using a MicroScan sidestream darkfield microscopy system (MicroVision Medical, Amsterdam, The Netherlands). Individual image files of 10-second length were obtained at each experimental time point and analyzed post hoc to obtain measurements of functional capillary density (FCD) (mm/mm^2) using Automated Vascular Analysis 3.2 software (MicroVision Medical, Amsterdam, The Netherlands). Only vessels less than 20 µm were used in the analysis to represent capillaries.

Brain Monitoring

A burr hole was drilled into the skull (10 mm paramedian of the coronal suture), and an intracranial pressure (ICP) catheter (Codman & Shurtleff, Raynham, MA) placed for continuous ICP and cerebral perfusion pressure (CePP) [CePP = MAP–ICP] monitoring. Cerebral electroencephalography (EEG) was continuously recorded with a swine montage of corkscrew scalp electrodes (Cascade Elite, Cadwell, Kennewick, WA) (13).

For continuous somatosensory evoked potential measurements, subdermal needle electrodes were placed bilaterally in a subfascial manner to provide bilateral median nerve stimulation. A current of 35 mA was delivered using 300-microsecond square wave pulses to elicit a response, with 300 stimulus responses averaged for each stored trial. SSEPs were recorded transcranially from the cortex using bipolar montages derived from EEG corkscrew scalp electrodes. SSEPs were also recorded from subcortical structures using corkscrew electrodes placed over the cervical spine and from the right brachial plexus from subdermal needle electrodes placed following supraclavicular neck dissection. Only SSEPs recorded in response to right forelimb stimulation were analyzed. SSEPs recorded in response to left forelimb stimulation were used as a control to confirm global ischemic mechanisms for left hemispheric signal change and to rule out focal causes. For baseline cortical SSEP measurements, animals were transitioned to total intravenous anesthesia (propofol, 1–12 mg/kg/hr) to enable comparison to post-CA values since inhalational anesthetics markedly attenuate cortical responses. Percent recovery was calculated in reference to the maximum baseline amplitude. Additionally, recovery amplitudes were dichotomized above and below 30% of maximum baseline amplitude since anything below this amplitude may be insignificant and/or only reflect electromyographic artifacts.

Cardiac Arrest Model

The experimental timeline is shown in **Figure 1**. After instrumentation was completed and baseline data were collected, ventricular fibrillation (VF) was induced by direct current (9 V) through a pacing wire and CA was confirmed by blood pressure tracing and ECG. Ventilation, anesthetics, fluids, and intraoperative warming devices were paused. Normothermic VF was left untreated for 8 minutes followed by 30 minutes of goal-directed CPR with asynchronous mechanical ventilation: tidal volume = 10 mL/kg, a respiratory rate equal to 10, F_{IO_2} equals to 1.0, PEEP equals to 5 cm H_2O. Manual CPR was performed with a personnel rotation every 2 minutes. If P_{ETCO_2} was less than 20 mm Hg, an impedance threshold device was added to the ventilation circuit. If P_{ETCO_2} remained less than 20 mm Hg, mechanical CPR was introduced using the Lund University Cardiac Assist System Chest Compression System (Jolife AB/Stryker Lund, Sweden). Epinephrine (0.015 mg/kg) was administered at minutes 12, 16, and 20 following CA with a vasopressin bolus (20 U) at minute 24.

Cannulation of the femoral vessels during CPR was conducted using a hybrid Seldinger technique through the groin incision using a 20–22 Fr venous cannula (Avalon Elite Multi-Port Venous Femoral Catheter, MAQUET, Rastatt Baden-Württemberg, Germany) for blood drainage and a 15–17 Fr arterial cannula (NovaLung, Xenios, Heilbronn, Germany) for blood reinfusion. Cannulae were secured and flushed with 10:1 heparinized saline while awaiting ECPR initiation. If P_{ETCO_2} values were less than 10 mm Hg for more than 10 minutes, ECPR was not initiated.

ECPR Protocol

The ECPR system was a roller peristaltic pump (COBE Perfusion System Precision Pump 043600-800;

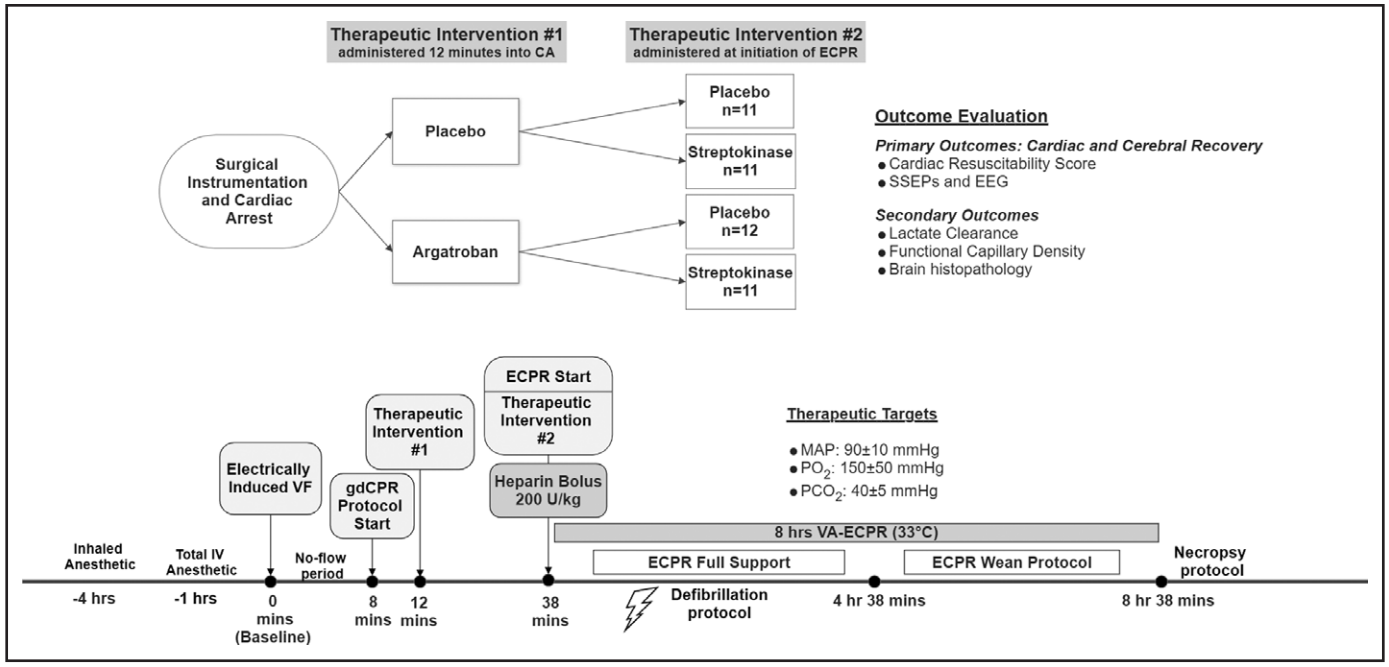

Figure 1. Study design and randomized groups. After 8 minutes of untreated ventricular fibrillation cardiac arrest, animals in all four groups underwent the same 32-minute goal-directed cardiopulmonary resuscitation (gdCPR) protocol. At minute 12 of cardiac arrest (CA), the animals received the first therapeutic intervention: placebo (20 mL of normal saline) or argatroban (350 μg/kg). In addition, at minutes 12, 16, and 20 all animals received epinephrine boluses (0.015 mg/kg). At minute 24, all animals received a vasopressin bolus (20 U). Extracorporeal cardiopulmonary resuscitation (ECPR) was initiated after 38 minutes, a heparin bolus of 200 U/kg was given, and the second therapeutic intervention was administered: placebo (50 mL of normal saline) or Streptokinase (1.5 MU). All four groups underwent the same defibrillation protocol (200 J x 3 shocks/hr) during the first 4 hr of ECPR support until the return of spontaneous heartbeat was achieved. If return of spontaneous heartbeat was achieved, a weaning protocol was followed during the remaining 4 hr of ECPR. Hemodynamic targets were maintained throughout ECPR with vasopressor support of epinephrine (0.1–0.5 μg/kg/min) and vasopressin (0–0.01 U/kg/min) during full extracorporeal membrane oxygenation (ECMO) support and norepinephrine (0.01–0.5 μg/kg/min) and dobutamine (2–40 μg/kg/min) during the ECMO weaning phase. At the end of the study, a necropsy was performed on all animals. Outcome evaluation focused on cardiac and cerebral recovery. EEG = electroencephalography, MAP = mean arterial pressure, SSEP = somatosensory-evoked potential, VF = ventricular fibrillation.

Terumo, Lakewood, CO). The circuit was primed with 2.0 L of plasmalyte, 500 mg of methylprednisolone sodium succinate, and 100 U/kg of heparin. ECPR was initiated 38 minutes after CA. ECPR was managed at targeted MAP greater than 65 mm Hg, with the following volume ventilation: tidal volume = 7–9 mL/kg, respiratory rate adjusted to maintain a $Paco_2 = 40 \pm 5$ mm Hg, $Fio_2 = 0.5$, and PEEP = 5 cm H_2o.

Epinephrine (0.1–0.5 μg/kg/min) and vasopressin (0–0.01 U/kg/min) were used for pressor support during the first 4 hours of ECPR. Activated clotting time (ACT) was maintained between 250 and 350 seconds. Electrolyte abnormalities were supplemented as needed throughout ECPR. After 15 minutes of ECPR, external electrical defibrillation was attempted for VF using up to three shocks at 200 J with a biphasic defibrillator (Philips Medical System, Andover, MA), and then repeated every 15 minutes if necessary.

In animals with return of spontaneous heartbeat (ROSB), weaning eligibility was assessed after 4 hours of ECPR. A progressive decrease in ECPR flow was performed maintaining a stable MAP greater than 65 mm Hg. Norepinephrine (0.01–0.5 μg/kg/min) and dobutamine (2–40 μg/kg/min) were used for hemodynamic support during ECPR weaning.

Blood Sampling

Periodic venous and arterial whole blood samples were collected for analysis throughout the study. Complete blood count and chemistry analysis were performed using ProCyte DX and Catalyst DX (Idexx Laboratories, Westbrook, ME). Coagulation parameters were assessed using the BCS XP coagulation analyzer (Siemens, Malvern, PA). Blood gas analysis was performed with the ABL800 Flex (Radiometer America, Brea, CA).

Histology

At the end of the study, the heart and brain were flushed with heparinized saline (10 U/mL), and perfusion fixed with 10% formalin for histologic analysis. Following the perfusion, the whole organs were immediately removed and placed in 10% formalin. After overnight fixation at 4°C, a brain matrix was used to cut the cerebrum into 5 mm coronal slices and the cerebellum into 5 mm sagittal slices. The free wall of each ventricle of the heart was sampled. The above specimens were immersed in phosphate-buffer saline-buffered 30% sucrose at 4°C for 5–7 days until they sank. All specimens were frozen rapidly in dry iced 2-methylbutane and stored at –80°C. The specimens were embedded with optimal cutting temperature compound (#23-730-571; Fisher HealthCare, Houston, TX) and cut into 20 µm sections by cryosectioning with Leica CM1860 (Leica Microsystems, Deerfield, IL). Brain sections were stained with cresyl violet (#AC229630050; Fisher Scientific, Fair Lawn, NJ), and myocardial sections were stained with hematoxylin and eosin (SH30-500 and 314-630; Fisher Scientific, Fair Lawn, NJ). All tissues were graded blindly using established grading scales. Five views under a 20× objective lens were randomly selected with microscopic system CTR6500 and LAS X software (Leica Microsystems, Deerfield, IL).

Experimental Design

Forty-eight swine (Yorkshire, 40–50 kg, 2–3 months old, evenly distributed sex) were studied. A white blood cell count of less than 20,000 cells/µL on experiment day was required for inclusion to avoid enrolling animals with active infection. Animals were block randomized into one of four groups: placebo + placebo (P + P), argatroban + placebo (ARG + P), placebo + streptokinase (P + STK), or argatroban + streptokinase (ARG + STK). Argatroban (350 µg/kg) or placebo (20 mL saline) was given at minute 12 of CA. STK (1.5 MU) or placebo (50 mL normal saline) was given at initiation of ECPR. Experimental operators were blinded to the intervention.

Outcomes

The primary cardiac recovery outcome was assessed 8 hours after ECPR using the cardiac resuscitability score (CRS) adapted from Spinelli et al (11). CRS is scored from 0 to 6 based on defibrillation (Yes = 1 or No = 0), ROSB (Yes = 1 or No = 0), weanability from ECPR (Yes = 1 or No = 0), and left ventricular systolic function after weaning (Normal or mildly depressed = 3, Moderately depressed = 2, Severely depressed = 1) (**Supplemental Table 1**, http://links.lww.com/CCX/B183). ROSB was defined as sustained evidence of organized ventricular contractions based on transthoracic echocardiography. Weaning was determined successful if a stable MAP greater than 65 mm Hg could be maintained with ECPR flow of less than 25%. The primary neurological outcome was measured as maximum percent recovery of the cortical SSEP response amplitude relative to pre-arrest baseline.

Secondary outcomes include FCD, myocardial and neuronal necrosis based on histologic analysis, and an 8-hour lactate clearance percentage. Mechanistic outcomes measured include assessment of coagulation parameters.

Statistical Analysis

Our power analysis was based on the primary outcomes of CRS (range 0–6). Setting alpha at 0.5, 12 subjects per group were estimated to provide power greater than 0.80 to detect a between-group difference as small as 1.0 point.

Variables are reported as mean followed by SD (in parentheses) unless otherwise noted. One-way, repeated-measure analysis of variance (ANOVA) tests were used to identify changes in individual variables over time. A Tukey-Kramer adjustment for multiple comparisons was used to maintain alpha at 0.05 in all cases. Primary and secondary outcomes were analyzed using the Kruskal-Wallis one-way ANOVA.

RESULTS

Among 48 animals, 3 animals were excluded due to adverse surgical complications unrelated to and independent of the outcome of the study criteria. Therefore, 45 animals (P + P, n = 11; ARG + P, n = 12; P + STK, n = 11; ARG + STK, n = 11) were analyzed. Baseline characteristics are shown in **Supplemental Table 2** (http://links.lww.com/CCX/B184). Physiologic and hemodynamic parameters during CPR were not statistically different between groups (**Fig. 2**). ECPR was successfully initiated for all animals. There was no statistical difference

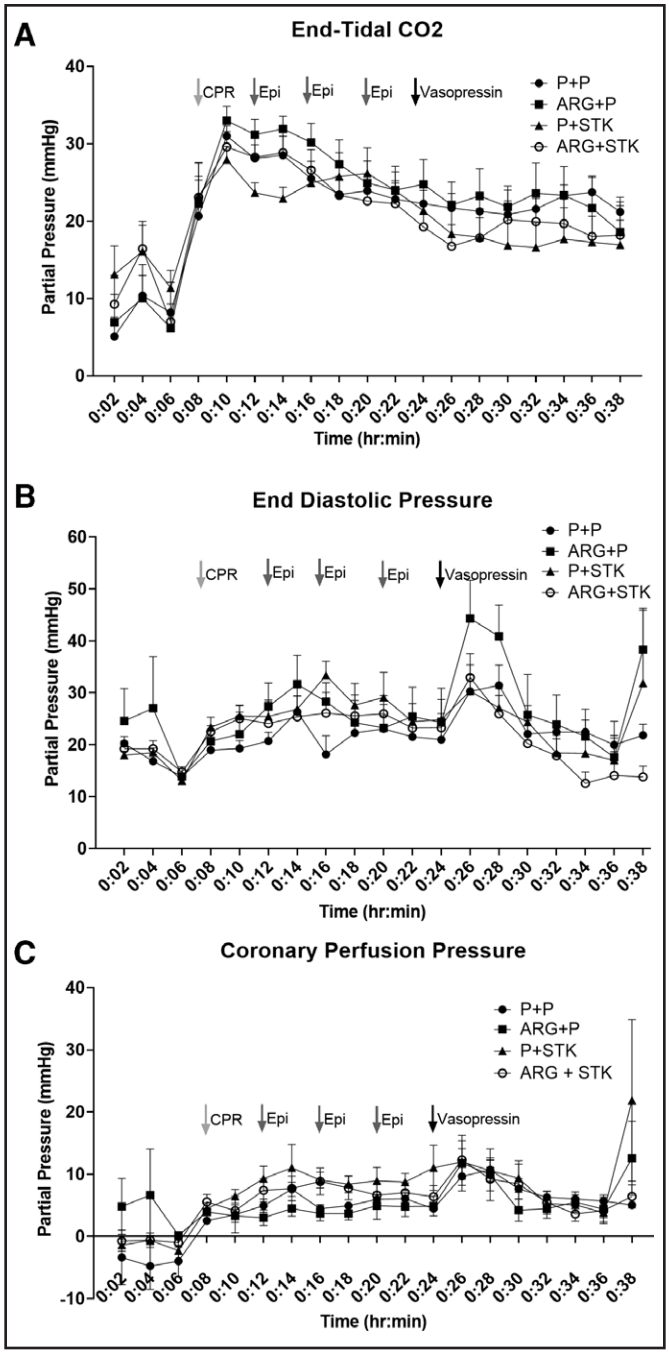

Figure 2. Physiologic and hemodynamic parameters during goal-directed cardiopulmonary resuscitation. **A**, End-tidal Co_2. **B**, End diastolic arterial pressure. **C**, Coronary perfusion pressure. *Arrows* represent cardiopulmonary resuscitation initiation and epinephrine and vasopressin doses given during resuscitation. Data are presented as mean with sem. Statistical analysis was performed using repeated measures analysis of variance with a significance set at $p < 0.05$ between groups. ARG = argatroban, CPR = cardiopulmonary resuscitation, Epi = epinephrine, P = placebo, STK = streptokinase.

in MAP, CePP, and ICP throughout ECPR between groups (**Fig. 3**).

Cardiac Recovery

Mean CRS of the four groups at the end of the experiment are summarized in **Table 1**. There were no statistical differences in CRS between groups (p = 0.16): P + P = 2.3 (1.0); ARG + P = 3.4 (2.1); P + STK = 1.6 (2.0); and ARG + STK = 2.9 (2.1). ROSB rates were (p = 0.26): P + P 11/11 (100%); ARG + P 11/12 (92%); P + STK 6/11 (55%); and ARG + STK 10/11 (91%). Rates of successful complete wean from ECPR were (p = 0.37): P + P 2/11 (18%); ARG + P 6/12 (50%); P + STK 1/11 (9%); and ARG + STK 3/11 (27%).

Neurological Recovery

There were no significant differences in the maximum percent of cortical SSEP amplitude recovery between the groups (p = 0.73): P + P = 23% (13%); ARG + P = 20% (13%); P + STK = 25% (14%); and ARG + STK = 26% (13%). The number of animals in each group to achieve greater than or equal to 30% amplitude recovery of the N20 was: P + P = 2; ARG + P = 3; P + STK = 2; and ARG + STK = 5 (Table 1; and **Supplemental Fig. 1**, http://links.lww.com/CCX/B180).

Secondary Outcomes

FCD initially decreased across all animals between baseline and ECPR onset [13.6 (2.38) μm^{-1} vs 11.3 (3.02) μm^{-1}, $p < 0.0001$]. FCD recovered to near baseline values in all groups, with no difference between baseline and 8 hours in any group, and no difference in percent recovery from baseline between groups (Table 1; and **Supplemental Fig. 2**, http://links.lww.com/CCX/B181). There were no significant differences between 8-hour lactate clearance among the groups (Table 1). There was also no significant difference in vasopressor or inotropic support between groups (**Supplemental Table 3**, http://links.lww.com/CCX/B185).

Histopathological Outcomes

The myocardial degeneration score was significantly lower in the ARG + STK group compared to the P + P group (***Fig. 4, A*** and ***C***). There was no significant difference in myocardial hemorrhage score between the treatment groups. The regional neuronal degeneration score in the caudate was significantly lower in the ARG + STK group compared to the P + P group

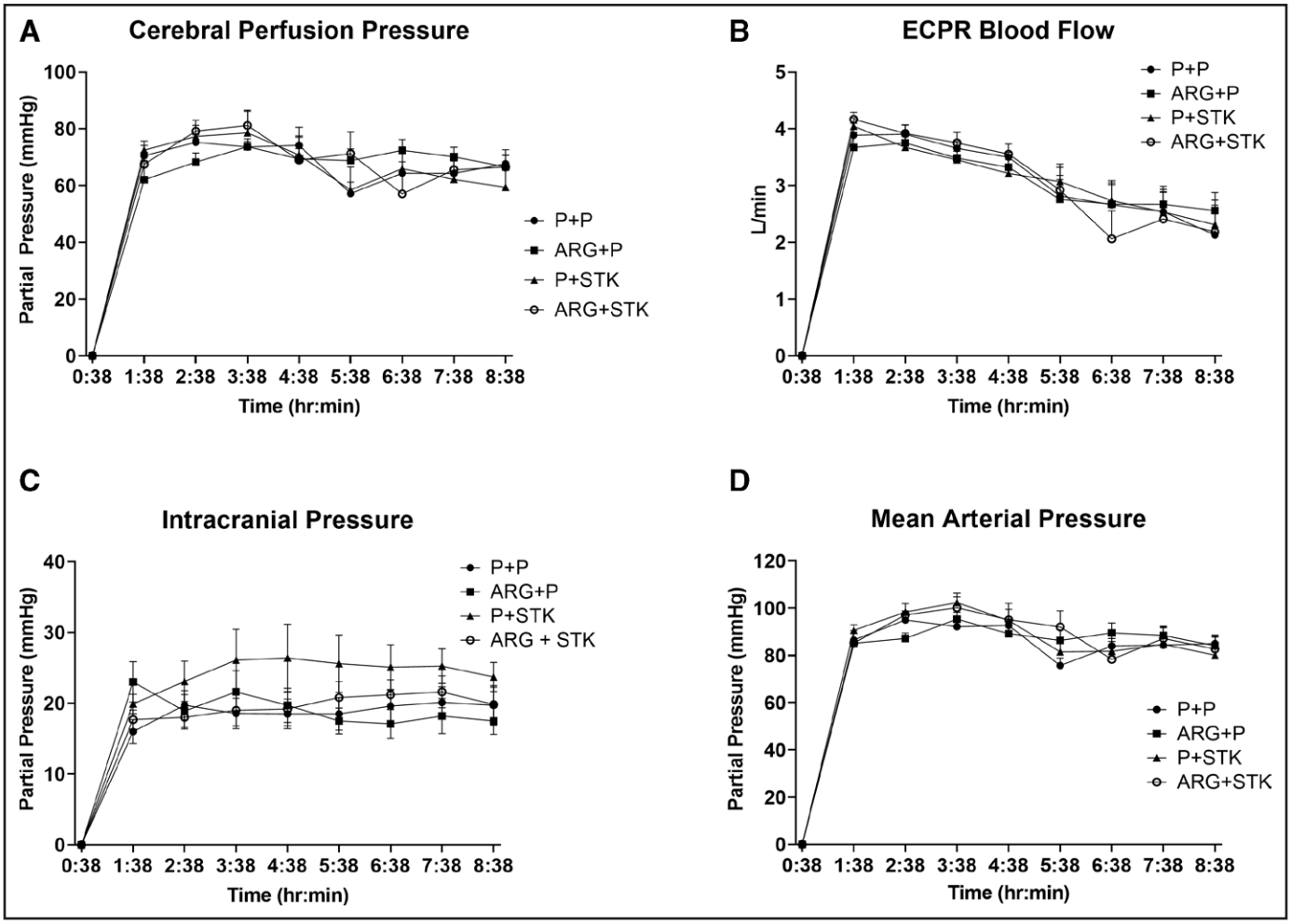

Figure 3. Hemodynamic parameters during extracorporeal cardiopulmonary resuscitation. **A**, Cerebral perfusion pressure. **B**, Extracorporeal cardiopulmonary resuscitation blood flow. **C**, Intracranial pressure. **D**, Mean arterial pressure. Data are presented as mean with SEM. Statistical analysis was performed using a repeated measures analysis of variance with a significance set at $p < 0.05$ between groups. ARG = argatroban, P = placebo, STK = streptokinase.

(*Fig. 4A*). Regional neurodegeneration scores were not different between groups in the cingulate cortex, hippocampus, or cerebellum.

Mechanistic Outcomes

This model demonstrated consumptive coagulopathy as shown by the significant decrease in average platelet count and fibrinogen levels and increase in D-dimer levels between baseline and at the end of ECPR (**Fig. 5**). However, there were no statistical differences in platelet count, D-dimers, or fibrinogen between groups before ECPR onset or at the end of the study. ACT before ECPR initiation (T38 minutes) was: P + P = 126.8 (29.5) seconds, ARG + P = 180.9 (32.3) seconds, P + STK = 141.8 (22.9) seconds, and ARG + STK = 186.6 (58.8) seconds. In terms of bleeding complications, there was no significant difference in total blood product transfusion requirement between groups (**Supplemental Fig. 3**, http://links.lww.com/CCX/B182).

DISCUSSION

We did not detect a significant benefit in our primary outcomes of short-term cardiac or neurologic recovery with early intra-arrest ARG and/or STK at onset of ECPR in our porcine model of prolonged out-of-hospital cardiac arrest (OHCA). Moreover, there were no significant differences in FCD or lactate clearance. However, histologic analysis showed evidence of reduced myocardial and neuronal necrosis with the two therapies combined compared to placebo.

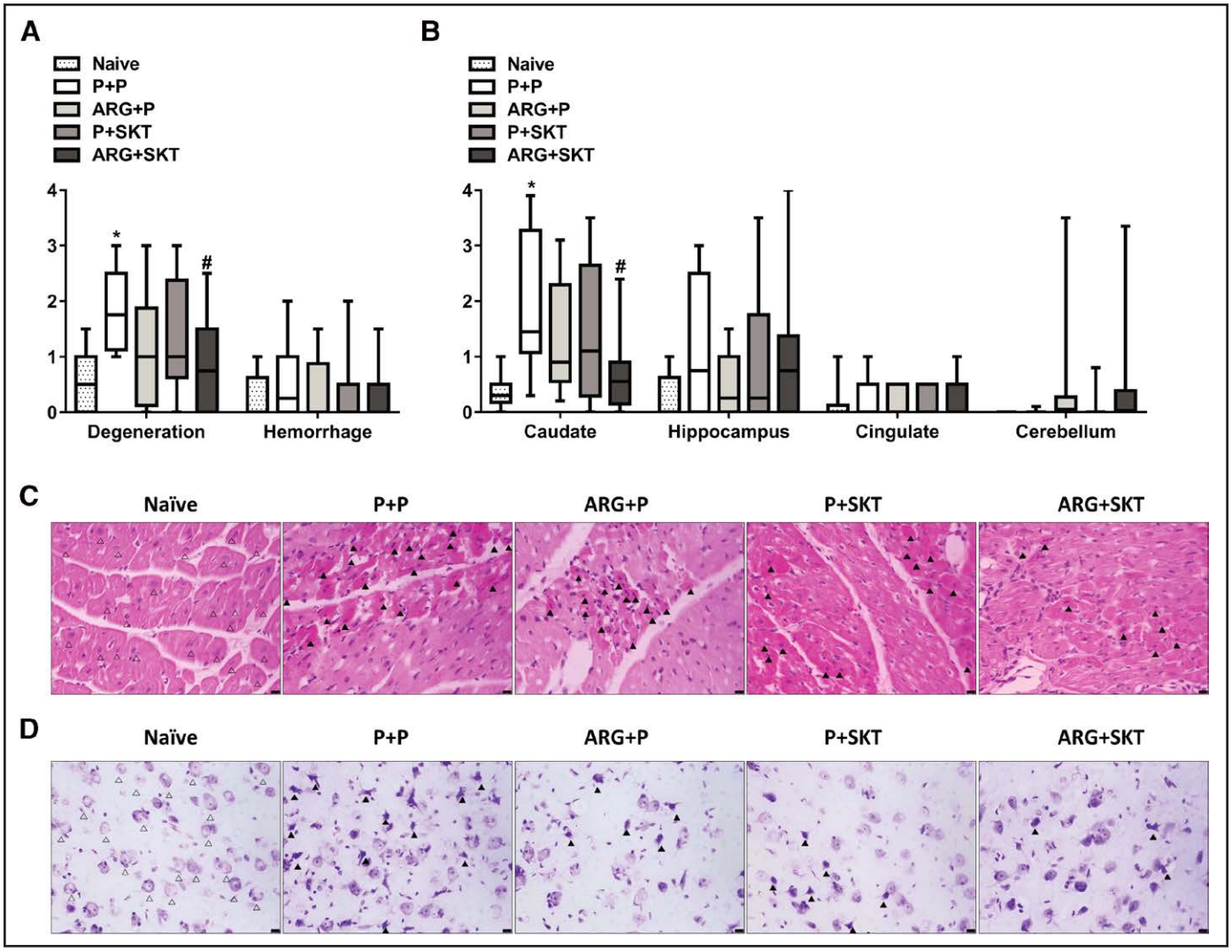

Figure 4. Histologic outcomes. Myocardial degeneration and myocardial hemorrhage (**A** and **C**) were assessed using hematoxylin and eosin-stained ventricle sections. The absence of degeneration was scored as 0. Mild degeneration (1 +) was classified as single to multiple foci of vacuolated, shrunken, or fragmented, hypereosinophilic staining. Moderate degeneration (2 +) was classified as more frequent multifocal foci of degeneration as described above, and severe degeneration (3 +) was defined as coalescing to regionally extensive involvement. Hemorrhage was graded on a similar severity scale. The absence of hemorrhage was scored as 0. Mild focal to multifocal hemorrhage was graded as a (1 +), moderate multifocal to regionally extensive hemorrhage was graded as (2 +), and more extensive hemorrhage involving large portions of the section was graded as (3 +). **A**, The data were plotted as box-whiskers, illustrating the medians, interquartile ranges, maximums, and minimums. The group medians were compared with the Mann-Whitney test. Naive: $n = 10$; placebo (P) +P: $n = 11$; argatroban (ARG) +P: $n = 12$; P+ streptokinase (STK): $n = 11$. $^{*}p < 0.01$ Naive vs P, $\#p < 0.05$ ARG + STK vs P. **C**, Representative images of hematoxylin and eosin-stained ventricles (magnification 400x). The *open arrowheads* showed normal cardiomyocytes and the *dark arrowheads* showed degenerated cardiomyocytes. Normal cardiomyocytes showed light and evenly eosinophilic staining with diffused hematoxylin-stained nuclei. In contrast, degenerated cardiomyocytes showed shrunken nuclei with hypereosinophilic cell bodies. Neurodegeneration (**B** and **D**) was assessed using cresyl violet-stained brain sections at the cingulate cortex, hippocampal CA1 sector, caudate, and cerebellum. Injured neurons were defined as dark-stained and shrunken with angular cell bodies, while the normal neurons were light stained, with a large cell body, round nucleus, and distinguishable nucleoli. Neurodegeneration was scored from 0 to 4 based on the percentage of degenerating neurons as 0: 0–5%, 1: 6%–25%, 2: 26%–50%, 3: 51–75%, and 4: 76%–100%. **B**, The data are presented as box-whisker plots, illustrating the medians, interquartile ranges, maximums, and minimums. The group medians were compared with the Mann-Whitney *U* test. Naive: $n = 10$; P+P: $n = 11$; ARG+P: $n = 12$; P+STK: $n = 11$; ARG+STK: $n = 11$. $^{*}p < 0.005$ Naive vs P, $\#p < 0.01$ ARG + STK vs P. **D**, Representative images of cresyl violet-stained caudate (magnification 400×) from each group. The *open arrowheads* showed the normal neurons and the *dark arrowheads* showed the degenerated neurons.

TABLE 1.
Primary and Secondary Outcomes

	P + P, ($n = 11$)	ARG + P, $n = 12$	P + STK, $n = 11$	ARG + STK, $n = 11$	p
Primary outcomes					
Cardiac					
Cardiac resuscitability score	2.3 (1.0)	3.4 (2.1)	1.6 (1.9)	2.9 (2.1)	0.16
Success of defibrillation, n[a]	11	11	6	10	–
Return of spontaneous heartbeat, n[b]	11	11	6	10	–
Weanability from extracorporeal cardiopulmonary resuscitation, n	3	6	2	3	–
Left ventricular ejection fraction after weaning, n					
Normal or mildly depressed	–	4	1	3	–
Moderately depressed	–	–	–	–	–
Severely depressed	2	1	1	–	–
Neurological					
N20 amplitude (% baseline)	23 (13)	20 (13)	25 (14)	26 (13)	0.73
N20 return > 30% of baseline, n	2	3	2	5	–
Secondary outcomes					
Lactate clearance (%)	33 (31)	40 (34)	27 (28)	33 (30)	0.84
Functional capillary density (% baseline)	94 (24)	85 (37)	83 (18)	92 (15)	0.82

ARG = argatroban, CA = cardiac arrest, P = placebo, STK = streptokinase.

The primary cardiac recovery outcome was measured as cardiac resuscitability score (CRS), which was based on our previous publication (11). The CRS ranges from 0 to 6 based on defibrillation (Yes = 1 or No = 0); return of spontaneous heartbeat (ROSB) (Yes = 1 or No = 0); weanability from extracorporeal cardiopulmonary resuscitation (Yes = 1 or No = 0); and left ventricular ejection fraction after weaning (Normal or mildly depressed = 3, Moderately depressed = 2, Severely depressed = 1). Defibrillation success was defined as the return of stable supraventricular rhythm. ROSB was assessed based on the evidence of heart contraction by qualitative echocardiography. The primary neurological outcome was measured as the maximum percent recovery of the porcine equivalent of the somatosensory-evoked potential N20 wave amplitude. Secondary outcomes include 8-hr lactate clearance (LaCl) [LaCl = (peak lactate concentration–final lactate concentration)/(peak lactate concentration × 100)]. Functional capillary density was measured using sublingual video of microcirculation of capillaries, analyzed, and reported as the maximum percent recovery of baseline. Values are mean (SD).

Since the hypercoagulable state of CA was first described by Crowell in 1955 (9), subsequent studies have reported mechanistic evidence for pathologic activation of the coagulation system during CA including: 1) increased fibrin monomers (14), 2) increased thrombin-antithrombin complexes (13, 14), 3) decreased fibrinogen (14), 4) decreased antithrombin III (AT III) (13), 5) increased activated protein C (13), and 6) decreased protein S (13). In this study, D-dimer levels in the placebo group were elevated from 0.5 mg/L at baseline to 4.4 ± 0.6 mg/L at 38 minutes after CA. The hypercoagulable state in our CA model is comparable to the clinical studies with CA duration of 19–20 minutes and D-dimer levels of 4–9 mg/L (13, 15). However, maximum D-dimer levels in the present study are likely underestimated since the maximum range of our assay was 5.0 mg/L.

For anticoagulant therapy, Ichinose et al (16), using a 15-minute CA model followed by ECPR in dogs, reported that pre-arrest 700 U/kg heparin administration with target ACT greater than 300 seconds resulted in a higher survival rate at 120 hours after ROSC compared to 200 U/kg heparin administration with target ACT around 150 seconds (100% vs 17%, $p < 0.05$).

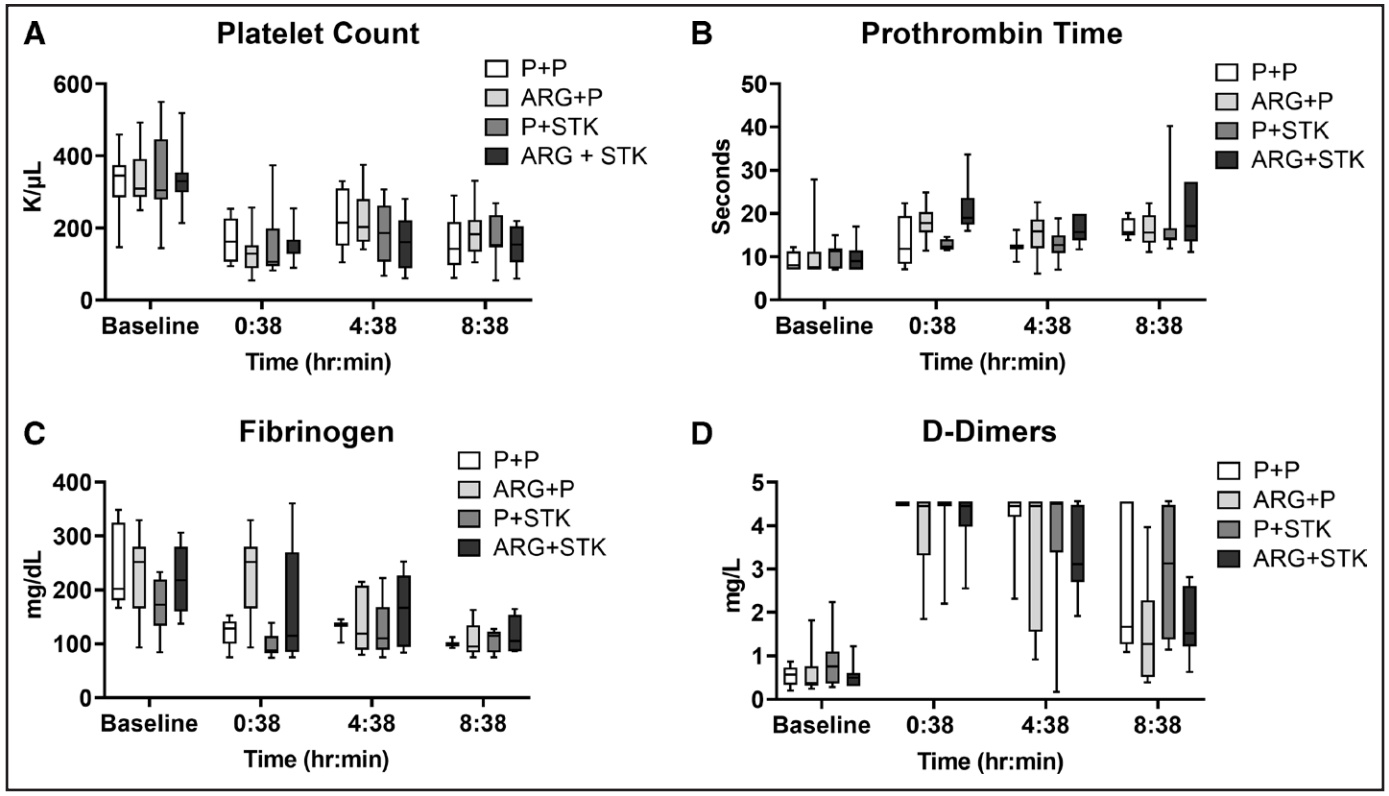

Figure 5. Coagulation parameters. **A**, Platelet count (K/µL). **B**, Prothrombin time (s). **C**, Fibrinogen (mg/dL). **D**, D-dimers (mg/L) during the course of the experiment are presented as box and whisker plots illustrating the medians, interquartile ranges, maximums, and minimums. Consumptive coagulopathy is demonstrated by the significant decrease in average platelet count and fibrinogen levels and increase in D-dimer levels between baseline and at the end of extracorporeal cardiopulmonary resuscitation ($p < 0.05$ for all groups). There were no significant differences between the groups at the end of each study for all coagulation parameters presented: platelet count: $p = 0.63$; prothrombin time: $p = 0.84$; fibrinogen: $p = 0.77$; D-dimer: $p = 0.19$. ARG = argatroban, P = placebo, STK = streptokinase.

ARG, a direct thrombin inhibitor, was selected as an alternative anticoagulant therapy to heparin in this study because: 1) AT III is depleted and unfractionated heparin (UFH) requires binding to AT for its anticoagulant activity (13), and 2) the UFH-AT complex cannot bind to thrombin (17). However, in the present study, the groups treated with ARG during CPR had an average ACT of 183.7 ± 46 seconds immediately before ECPR onset. Thus, it is possible that the ARG dose of 350 µg/kg was suboptimal for preventing microvascular thrombosis in this CA model.

For thrombolytic treatment, Crowell was the first to demonstrate the benefit of pretreating animals with STK before CA (18). The thrombolysis in CA Thrombolysis in Cardiac Arrest trial did not show the benefit of tenecteplase during CPR in OHCA (19). The absence of a treatment effect in this study may have been due to a short "no-flow" or total arrest period that affected the development of microvascular thrombosis. Our previous study showed that 1 MU of STK improved recovery of cardiac function in a porcine model with 30 minutes of untreated CA followed by ECPR (11). In the present study, we could not detect the benefit of STK on functional recovery despite the higher dose (1.5 MU). The discrepancy between our previous study and the present study could potentially be caused by a greater burden of microvascular thrombosis with 30 minutes of no-flow compared to 8 minutes of no-flow followed by 30 minutes of CPR. Fischer et al (6), using a cat model with CA at 5, 15, or 30 minutes of untreated VF followed by 5 minutes of open chest CPR observed 7%, 30%, and 65% no-reflow in the forebrain respectively at 30 minutes after ROSC.

Although we did not detect a treatment effect on our primary functional outcomes or secondary physiologic outcomes, the histologic analysis provided evidence of reduced myocardial and neuronal necrosis in animals that received both ARG and STK. Of the four brain regions examined, reduced neurodegeneration was only statistically significant in the caudate region.

However, neurodegeneration was limited in the other brain regions at the time point examined limiting the feasibility of detecting a significant treatment effect in those regions. Since no-reflow is likely to cause early tissue necrosis, these results do provide histologic evidence supporting the study hypothesis.

LIMITATIONS

Our study has several limitations. Most importantly we did not measure long-term outcomes, and therefore could have missed important benefits in recovery of cardiac and neuronal function after 8 hours of ECPR. Second, the burden of microvascular thrombus may not have been severe enough in this model for the therapies to have a detectable benefit. Longer durations of total CA before ECPR are common in human studies. We also did not conduct a dose-response study to optimize the dose of each therapy, therefore the interventions may not have had the optimal treatment effect. Finally, additional or alternative causal mechanisms of the no-reflow phenomenon have been proposed that might require alternative therapeutic strategies (20, 21).

CONCLUSIONS

In this porcine model of prolonged CA, early anticoagulation during CPR and/or thrombolytic therapy at the initiation of ECPR did not improve early recovery of heart and brain function. Limited histologic evidence of reduced myocardial and neuronal injury was observed when the two therapies were combined, suggesting the need for further investigation using long-term outcomes.

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REFERENCES

1. Holmberg MJ, Geri G, Wiberg S, et al; International Liaison Committee on Resuscitation's (ILCOR) Advanced Life Support and Pediatric Task Forces: Extracorporeal cardiopulmonary resuscitation for cardiac arrest: A systematic review. *Resuscitation* 2018; 131:91–100
2. Inoue A, Hifumi T, Sakamoto T, et al; SAVE-J II study group: Extracorporeal cardiopulmonary resuscitation in adult patients with out-of-hospital cardiac arrest: A retrospective large cohort multicenter study in Japan. *Crit Care* 2022; 26:129
3. Bartos JA, Grunau B, Carlson C, et al: Improved survival with extracorporeal cardiopulmonary resuscitation despite progressive metabolic derangement associated with prolonged resuscitation. *Circulation* 2020; 141:877–886
4. Belohlavek J, Smalcova J, Rob D, et al; Prague OHCA Study Group: Effect of intra-arrest transport, extracorporeal cardiopulmonary resuscitation, and immediate invasive assessment and treatment on functional neurologic outcome in refractory out-of-hospital cardiac arrest: A randomized clinical trial. *JAMA* 2022; 327:737–747
5. Fischer M, Böttiger BW, Popov-Cenic S, et al: Thrombolysis using plasminogen activator and heparin reduces cerebral no-reflow after resuscitation from cardiac arrest: An experimental study in the cat. *Intensive Care Med* 1996; 22:1214–1223
6. Fischer M, Hossmann KA: No-reflow after cardiac arrest. *Intensive Care Med* 1995; 21:132–141
7. del Zoppo GJ, Schmid-Schönbein GW, Mori E, et al: Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. *Stroke* 1991; 22:1276–1283
8. Ge L, Zhou X, Ji WJ, et al: Neutrophil extracellular traps in ischemia-reperfusion injury-induced myocardial no-reflow: Therapeutic potential of DNase-based reperfusion strategy. *Am J Physiol Heart Circ Physiol* 2015; 308:H500–H509

9. Crowell JW, Sharpe GP, Lambright RL, et al: The mechanism of death after resuscitation following acute circulatory failure. *Surgery* 1955; 38:696–702
10. Lewis BE, Matthai WH Jr, Cohen M, et al; ARG-216/310/311 Study Investigators: Argatroban anticoagulation during percutaneous coronary intervention in patients with heparin-induced thrombocytopenia. *Catheter Cardiovasc Interv* 2002; 57:177–184
11. Spinelli E, Davis RP, Ren X, et al: Thrombolytic-enhanced extracorporeal cardiopulmonary resuscitation after prolonged cardiac arrest. *Crit Care Med* 2016; 44:e58–e69
12. Kerut EK, Valina CM, Luka T, et al: Technique and imaging for transthoracic echocardiography of the laboratory pig. *Echocardiogr* 2004; 21:439–442
13. Adrie C, Monchi M, Laurent I, et al: Coagulopathy after successful cardiopulmonary resuscitation following cardiac arrest: Implication of the protein C anticoagulant pathway. *J Am Coll Cardiol* 2005; 46:21–28
14. Böttiger BW, Motsch J, Böhrer H, et al: Activation of blood coagulation after cardiac arrest is not balanced adequately by activation of endogenous fibrinolysis. *Circulation* 1995; 92:2572–2578
15. Buchtele N, Schober A, Schoergenhofer C, et al: Added value of the DIC score and of D-dimer to predict outcome after successfully resuscitated out-of-hospital cardiac arrest. *Eur J Intern Med* 2018; 57:44–48
16. Ichinose K, Okamoto T, Tashiro M, et al: The effects of pre-arrest heparin administration dose for cardiac arrest model using extracorporeal lung and a heart assist (ECLHA) in dogs. *Resuscitation* 2006; 69:311–318
17. Weitz JI, Hudoba M, Massel D, et al: Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. *J Clin Invest* 1990; 86:385–391
18. Crowell JW, Smith EE: Effect of fibrinolytic activation on survival and cerebral damage following periods of circulatory arrest. *Am J Physiol* 1956; 186:283–285
19. Böttiger BW, Arntz HR, Chamberlain DA, et al; TROICA Trial Investigators: Thrombolysis during resuscitation for out-of-hospital cardiac arrest. *N Engl J Med* 2008; 359:2651–2662
20. Daniele SG, Trummer G, Hossmann KA, et al: Brain vulnerability and viability after ischaemia. *Nat Rev Neurosci* 2021; 22:553–572
21. Brixius SJ, Pooth JS, Haberstroh J, et al: Beneficial effects of adjusted perfusion and defibrillation strategies on rhythm control within controlled automated reperfusion of the whole body (CARL) for refractory out-of-hospital cardiac arrest. *J Clin Med* 2022; 11:2111