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Emergency Ultra-Deep Hypothermia in Cardiac Arrest Induced by Blood Loss (Experimental Study on Nonhuman Primates)

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Summary

The survival rate of critically injured individuals with severe blood loss and cardiac arrest is close to zero.

Aim. To evaluate the feasibility of using emergency ultra-deep hypothermia (EUDH) in an experimental model of cardiac arrest induced by blood loss in nonhuman primates.

Materials and Methods. Five male olive baboons (*Papio anubis*), weighing 19.8 (18.8–23.8) kg, were subjected to severe blood loss leading to cardiac arrest. After 1 minute of observation and 3 minutes of cardiopulmonary resuscitation (CPR), aortic arch cooling was initiated using extracorporeal membrane oxygenation (ECMO) with a 4°C solution to achieve a nasopharyngeal temperature of 10°C. Whole-body cooling followed until a rectal temperature of 16°C was reached. Balloon catheters were used to disconnect the upper and lower halves of the body. Once the target temperatures were reached, the ECMO circuit was turned off and an open laparotomy was performed to simulate damage control strategies. One hour after cardiac arrest, slow rewarming began at a rate of 1°C per 10 minutes to 1°C per hour, accompanied by reinfusion of previously collected blood. After return of spontaneous circulation (ROSC), sustained breathing, and tracheal extubation, the animals were transferred to a vivarium.

Results. During deep hypothermia, cerebral oximetry values remained within normal limits in all animals. Sustained ROSC was recorded in 4 of 5 animals at temperatures between 22–25°C. Two animals survived to the end of the experiment but died after extubation, 44 and 19 hours after the start of the experiment. Cooling rates for survivors were 7–11 minutes compared to 23–37 minutes for non-survivors. Causes of death included systemic hypoperfusion with subsequent reperfusion syndrome as evidenced by progressive lactate elevation, elevated creatine phosphokinase levels, cerebral edema, myocardial ischemia, and transient coagulopathy.

Conclusion. EUDH supports adequate cerebral perfusion during temporary circulatory arrest. Recovery of cardiac activity and, in some cases, awakening are achievable during the rewarming phase. Causes of death and possible corrective measures require further investigation.

Keywords: ultra-deep hypothermia; cardiac arrest; blood loss; reperfusion; primate experiment; ECMO

Conflict of Interest. The authors declare no conflict of interest.

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Introduction

Severe trauma with ongoing bleeding and the development of cardiac arrest is overwhelmingly fatal. Current resuscitation methods are largely ineffective in traumatic cardiac arrest (TCA). The success rate of standard cardiopulmonary resuscitation (CPR), including closed chest compressions, is extremely low [1, 2]. Even when thoracotomy is performed to place an aortic cross-clamp and perform open chest cardiac massage, survival rates do not exceed 10% [3].

Regardless of the cause of cardiac arrest, irreversible changes begin to occur in the brain within 5 minutes and in the heart within 20 minutes [4, 5]. Since the 1950s and 1960s, researchers have sought ways to reduce the oxygen demand of vital organs [6, 7]. Building on the pioneering work of V. A. Negovsky, who studied mechanical circulatory support and the use of hypothermia [5, 8, 9], P. Safar and S. Tisherman introduced the concept of emergency preservation and resuscitation (EPR) [10–12].

The essence of the method is emergency profound (ultra-deep) hypothermia (EPH), which is achieved by rapid cooling of the upper body (head and heart) followed by whole body cooling to a temperature of 10–16°C. This is achieved by infusing cooled (4°C) saline into the vascular system. According to its proponents, this approach minimizes oxygen demand to vital organs, allowing surgeons to perform life-saving surgery followed by resuscitation and gradual rewarming.

EPR has been successfully implemented in animal studies with high survival rates [13, 14], paving the way for regulatory approval of a pilot clinical trial in trauma patients [15].

The described method has several limitations. It requires a wide thoracotomy approach to clamp the major vessels (aorta and inferior vena cava [IVC]) and to place the return cannula (directed toward the heart in the descending aorta) and the drainage cannula (in the right atrial appendage). This creates an isolated «upper» circuit for rapid cooling of the brain. However, one of the critical tasks of EPH — rapid cannulation and isolation of the upper circuit — is difficult to achieve with this protocol. Thoracotomy and open cannulation of major vessels can take more than 5 minutes, which is critical to maintaining cerebral perfusion. In addition, the extensive thoracotomy itself introduces another source of blood loss due to the subsequent development of coagulopathy.

It was hypothesized that the principles of EPH could be implemented in a minimally invasive manner. This could be achieved by combining resuscitative endovascular balloon occlusion of the aorta (REBOA) with open cannulation of the right brachial artery, allowing selective perfusion of the aortic arch [16].

The aim of this study was to experimentally evaluate the feasibility of using a modified EPH technique during hemorrhagic shock-induced cardiac arrest in non-human primates.

Materials and Methods

The study was conducted at the Kurchatov Medical Primatology Complex of the National Research Center «Kurchatov Institute» (MPC NRC «Kurchatov Institute»), Sochi, with the approval of the local ethics committee (Protocol No. 279, dated June 27, 2023).

The study included five male olive baboons (*Papio anubis*) with a mean age of 5.6 years (range: 5.3–10.8 years) and a mean body weight of 19.8 kg (range: 18.8–23.8 kg). The selection of these animals (non-human primates) was driven by the need to evaluate the development of functional, including cognitive, impairments resulting from macro- and/or microstructural changes in the brain under conditions of deep hypothermia. All procedures involving baboons were performed in accordance with generally accepted legal and ethical standards for animal care, according to standard operating procedures adopted at the MPC NRC «Kurchatov Institute». Compliance with the Decision of the Council of the EEC dated November 3, 2016, No. 81 «On Approval of the Rules of Good Laboratory Practice for the Eurasian Economic Union in the Field of Medicinal Products Handling», as well as GOST 33044–2014 «Principles of Good Laboratory Practice» and GOST 33218–2014 Interstate Standard «Guidelines for the Housing and Care of Laboratory Animals. Rules for housing and care of non-human primates» was ensured.

The experimental protocol consisted of several key phases: animal preparation, preparation and priming of the ECMO circuit, simulation of blood loss and cardiac arrest, emergency hypothermia, and subsequent rewarming of the animal (Fig. 1).

Animal Preparation and Vascular Catheterization. After 24 hours of fasting, animals were sedated with intramuscular tiletamine and zolazepam (Telazol, Zoetis®, USA) at a dose of 15–20 mg/kg. They were transported to the operating room where tracheal intubation was performed followed by mechanical ventilation. Animals were positioned supine on the operating table with limbs extended. A 5 Fr introducer was placed in the left jugular vein to facilitate maintenance of intravenous fluid therapy and blood sampling. Both femoral arteries were catheterized with 7 Fr introducers: one for invasive BP monitoring and placement of a balloon catheter for thoracic aortic occlusion (MIT catheter, Zheleznodorozhny, Russia) and the other for insertion of a 12 Fr arterial return cannula.

In addition, the right femoral vein was catheterized to insert a second balloon catheter to occlude the inferior vena cava (IVC) at its junction with the right atrium, ensuring complete isolation of the upper circulatory compartment.

A 5 cm incision was made in the right axilla to expose the right brachial artery and vein. Through these, a second 15 Fr arterial return cannula was inserted with its tip positioned in the aortic arch, and a 17 Fr drainage cannula was inserted with its tip positioned in the superior vena cava (SVC) lumen (all cannulae from Biomedicus, Medtronic, USA). To prevent ischemia, a 20 G peripheral catheter was placed as a distal shunt in the artery of each catheterized limb. All intravascular procedures were

performed under fluoroscopic guidance using a C-arm system.

Temperature in the upper and lower half of the body was measured with two sensors placed in the nasopharynx (T_n) and rectum (T_r). In addition, brain, abdominal cavity, and limb temperatures were assessed using radiothermometry (RTM-01-RES, Russia) at a measurement depth of 4–6 cm. Cerebral perfusion quality was monitored using the Invos™ 5100C cerebral/somatic oximeter equipped with Somasensor technology (Medtronic, USA). This device uses near-infrared spectroscopy to determine tissue oxygen saturation (StO_2). Intracranial pressure (ICP) was monitored through a trepanation hole in the left hemicranium, where a parenchymal sensor was placed prior to systemic heparinization (Spiegelberg, Germany). A catheter was placed in the bladder to monitor urine output.

Prior to cannulation, systemic heparinization was performed by administering 70–90 IU of heparin per kilogram of body weight (Heparin Sodium Braun, B. Braun, Germany) to achieve an activated clotting time (ACT) of at least 350 seconds (Actalyke Mini II, Helena Lab., USA).

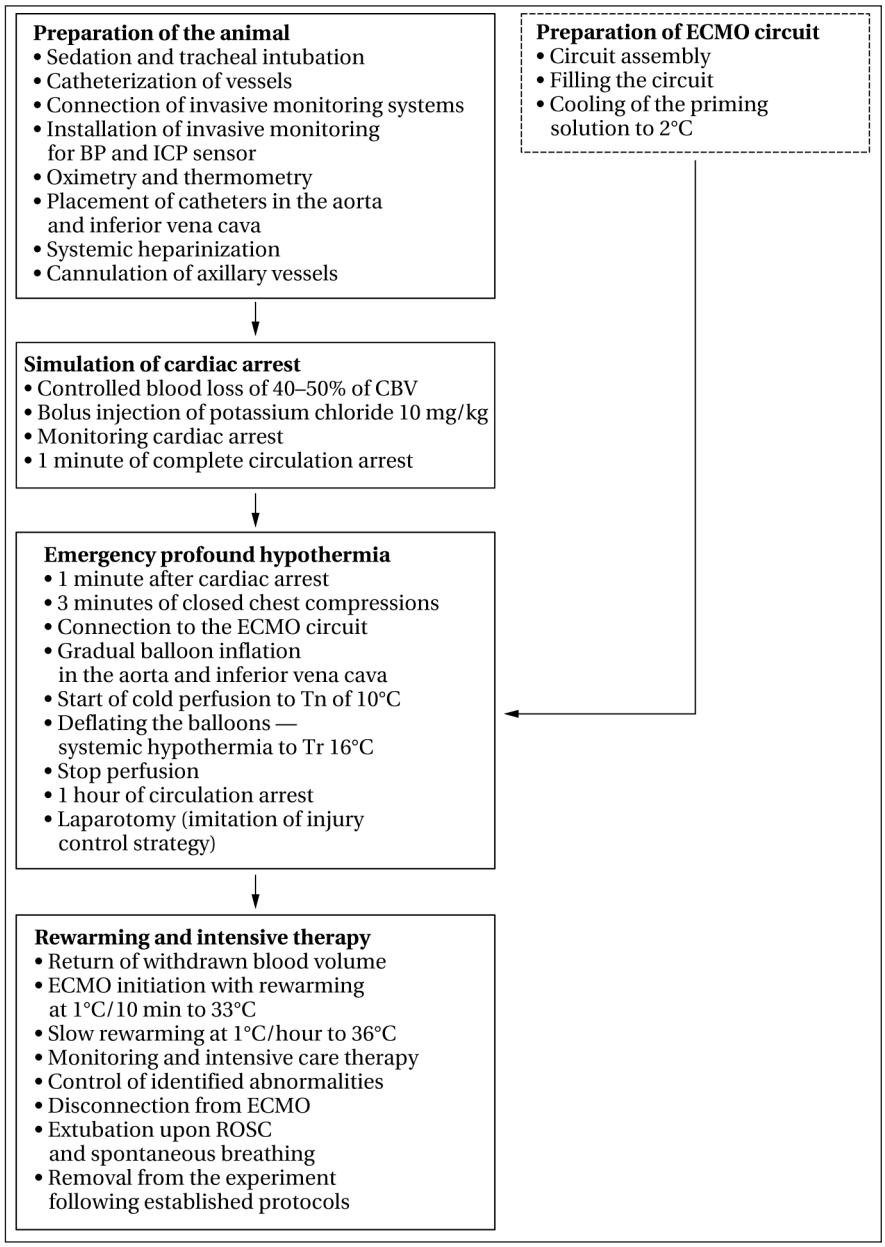

Fig. 1. Experimental protocol for evaluating the efficacy of emergency profound hypothermia in non-human primates.

Note. BP — blood pressure; ROSC — return of spontaneous circulation; ICP — intracranial pressure; CBV — circulating blood volume; T_n — nasopharyngeal temperature; T_r — rectal temperature.

ECMO circuit preparation. The ECMO circuit was preassembled before the procedure and consisted of an RF-36 centrifugal pump head connected to a portable ECMO device (ExStream, Transbiotech, Russia) and an oxygenator (Quadrox-i, Maquet Cardiopulmonary GmbH, Germany). The priming solution for the circuit contained the following components:

— 5 parts Gelofusine (B. Braun, Germany)

— 5 parts Sterofundin (B. Braun, Germany)

— 2 parts 4% sodium bicarbonate solution (Dalchimpharm, Russia)

— 1 part mannitol (Biosynthesis, Russia)

— 1 part 20% albumin (Microgen, Russia)

In addition, 20 mL of 40% glucose and 20 mL of 4% potassium chloride were added to the circuit. The prepared circuit was closed, deaerated, and placed in standby mode for connection. During this time, the prime solution was cooled to 4°C using a thermoregulation unit (TRU, 3T, Sorin Stockert, Germany) connected to the oxygenator.

Simulation of blood loss and cardiac arrest. Controlled blood loss of 40–50% of the circulating blood volume (CBV) was simulated by slowly (over 30–45 minutes) withdrawing venous blood with a syringe from the femoral vein introducer. Blood was collected in containers containing CPDA-1 anticoagulant for reinfusion during the rewarming phase.

When systolic blood pressure (SBP) dropped below 40 mmHg, blood withdrawal was accelerated

and 4% potassium chloride solution at a dose of 10 mg/kg was administered to induce cardiac arrest. Although not a standard approach, the addition of potassium chloride facilitated rapid cardiac arrest and creation of a timepoint while producing systemic hyperkalemia to prevent early spontaneous rhythm recovery during subsequent rewarming. Cardiac arrest was monitored by ECG, invasive blood pressure measurements, and ultrasound.

Emergency profound hypothermia (EPH). One minute after arrest, chest compressions were initiated and continued for 3 minutes. During this time, the ECMO circuit lines were disconnected and connected to the axillary venous drainage and arterial return cannulae. CPR was then stopped and the EPH resuscitation protocol was initiated. The aortic balloon was inflated, the clamps on the ECMO lines were removed, and perfusion of the upper body with cold prime solution was initiated (Fig. 2, *a*). To accelerate cooling and maintain the target T_n, ice packs were also placed around the head.

After three minutes of cooling, a balloon was inflated in the inferior vena cava (IVC) to isolate the «upper» circulation and accelerate cooling. When the brain temperature reached 10°C, the balloons were deflated and removed, and the clamp on the additional arterial return cannula in the femoral artery was released, initiating perfusion of the lower body (Fig. 2, *b*). External cooling with ice packs placed around the animal's body was added to the invasive cooling.

After cooling the brain to 10°C and the body to 16°C, artificial circulation and mechanical ventilation were suspended for 1 hour. A laparotomy was performed, carefully avoiding damage to internal organs, to simulate a surgical procedure for hemorrhage control. Cooling elements were then placed in the abdominal cavity, which was subsequently closed using a continuous suture.

Rewarming and postoperative monitoring. After 60 minutes, perfusion and mechanical ventilation were resumed, with the animal's previously collected blood added to the ECMO circuit while blood gas abnormalities identified during analysis were corrected. External cooling was discontinued and a relaparotomy was performed to remove the cooling elements. Gradual rewarming was performed with a temperature regulating unit (TRU), maintaining a temperature gradient of no more than 4°C between the circuit and nasopharyngeal temperature (T_n). Rewarming was performed at a rate of 1°C every 10 minutes until 33°C was reached, after which the rate was slowed to 1°C per hour. Once body temperature reached 36°C, passive slow rewarming was

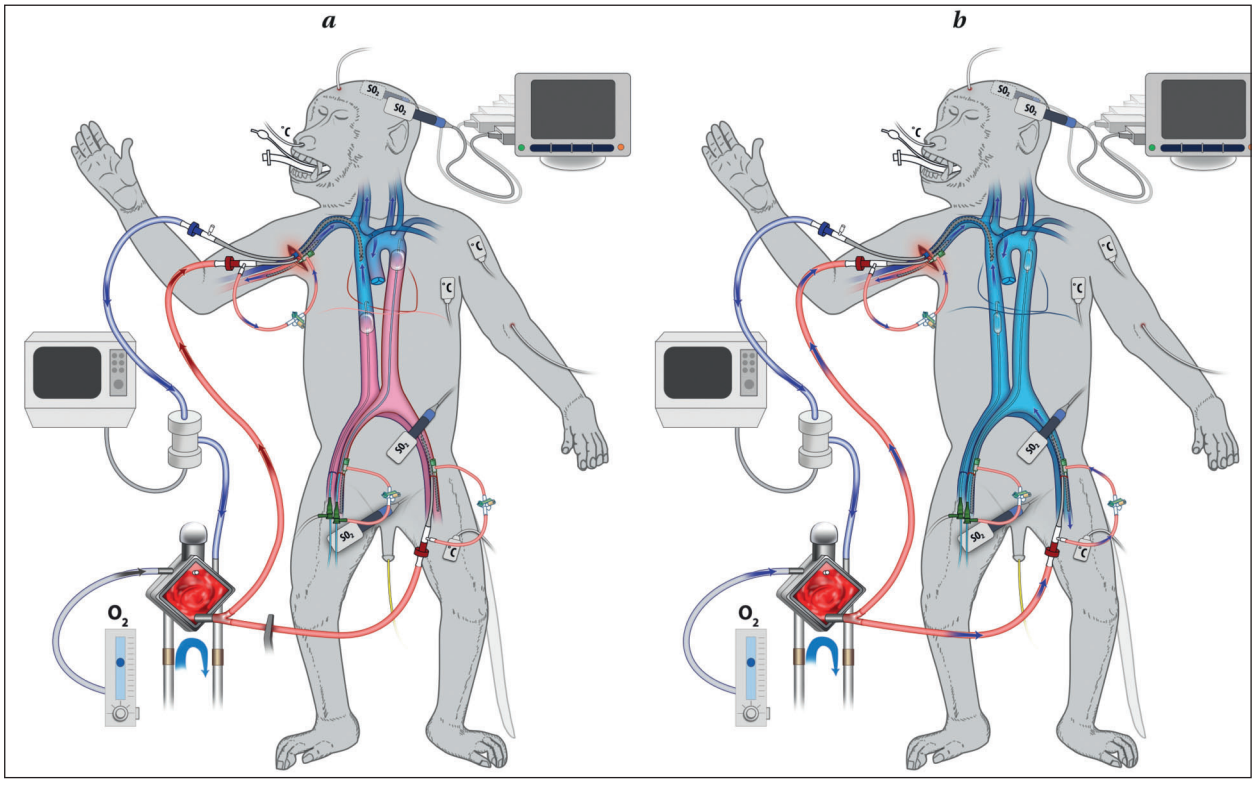

Fig. 2. Graphical representation of the experiment
Note. *a* — initiation of the emergency profound (ultra-deep) hypothermia protocol using a portable ECMO machine. Blood is removed from the superior vena cava and returned to the aortic arch. To isolate the upper circuit for rapid cooling of the brain, a balloon catheter inserted via the femoral artery is inflated at the level of the aortic arch, and an additional balloon is inflated in the inferior vena cava at the level of the caval opening. *b* — both balloons are deflated to allow cooling of the entire body. An additional return cannula in the femoral artery is activated to increase systemic cooling efficiency.

used until normothermia was achieved. The perfusion index was gradually increased from 1.0 (at T_n 10–15°C) to 1.5 (at T_n 15–20°C), 2.0 (at T_n 20–30°C), 2.5 (at T_n 30–35°C), and up to 3.0 at normothermia.

Norepinephrine was administered via an infusion pump to correct systemic hypotension, and adrenaline was added if higher doses were ineffective. Additional infusions of gelofusine, 20% albumin, sodium bicarbonate, and mannitol were given as needed to maintain the calculated flow volume. Furosemide was administered to stimulate diuresis when indicated, and rapid-acting insulin (Actrapid, Novo Nordisk, Denmark) was used to correct hyperglycemia. Sodium heparin was used to prolong the activated clotting time (ACT), and calcium chloride was administered to correct hypocalcemia (serum calcium $<$ 1 mmol/L). If no urine output was observed, an ultrafiltration column (Diacap Pro, B. Braun, Germany) was connected to the ECMO circuit to correct hyperhydration.

In the event of coarse ventricular fibrillation during rewarming, defibrillation was performed by delivering a 100–150 J shock with a Lifepak 12 defibrillator (Medtronic, USA).

Once the T_n reached 37°C and the animal was stabilized with a sustained spontaneous breathing pattern and limb movement, endotracheal extubation was performed. The animal was then returned to the vivarium for further observation. In the absence of spontaneous cardiac activity and respiration after complete rewarming, biological death was confirmed, and the animal was euthanized by exsanguination under general anesthesia.

The primary endpoint was return of spontaneous circulation (ROSC), while secondary endpoints included 24-hour survival and recovery from anesthesia.

Blood biochemical parameters were measured spectrophotometrically using a Chemray 240 automated analyzer (Rayto, China). Lactate levels were assessed by direct amperometric analysis using a GemPremier 3500 (IL Werfen, USA), which was also used for blood gas composition and acid-base status analysis.

Tissue samples for histologic analysis were fixed in 10% neutral buffered formalin (pH 7.4). Standard histologic processing was performed followed by paraffin embedding (Histomix, BioVitrum LLC, St. Petersburg, Russia). Paraffin blocks were cut at 4 μm and stained with hematoxylin and eosin. Morphological analysis was performed with an Axio Lab.A1 biological microscope (Carl Zeiss Microscopy GmbH, Germany), and microphotographs were taken with an Axiocam 105 color digital camera (Carl Zeiss Microscopy GmbH, Germany).

The identified changes in tissues and internal organs at both macroscopic and microscopic levels were thoroughly described and documented in the protocols of pathological anatomical examination.

Data collection and registration were performed using Excel (Microsoft, USA). Statistical analysis and graphing were performed using Prism 10.0 (GraphPad, USA). Due to the lack of a comparison group, no comparative analysis of the samples was performed. Quantitative variables (including those in Fig. 3) were presented as median with interquartile range.

Results and Discussion

In all five cases, the proposed TCA model was successfully implemented, achieving a brain temperature of 10°C and a body temperature of 16°C. During controlled blood loss, 600 (400–760) ml of blood was collected and prepared for subsequent reinfusion. The mean time for brain cooling was 23 (9–36) minutes. The maximum possible time to cool the brain to 10°C was 7 minutes. The variability in cooling rate was directly related to the flow in the ECMO circuit. When the walls of the vein collapsed during blood withdrawal from the vena cava superior, the flow decreased, which slowed the entire cooling process.

During the gradual rewarming, the T_n and T_r gradually converged and began to rise (Fig. 3, *a*). When the nasopharyngeal temperature reached 18°C, spontaneous electrical activity of the heart was observed (initially, the rhythm was not sinus). When the temperature reached 22–25°C, the first spontaneous cardiac contractions occurred, often progressing to ventricular fibrillation (VF). Considering VF to be potentially damaging to the myocardium, these episodes were managed by administering additional potassium chloride. Sinus rhythm was restored when the T_n reached approximately 28–30°C. In two cases, this required 1–2 defibrillator shocks at 120–150 J. In one of the five animals (No. 1), sustained ROSC was not achieved despite the appearance of rhythm and spontaneous cardiac contractions, and the experiment was terminated prematurely after 13 hours of rewarming. Thus, ROSC was successfully achieved in 4 out of 5 animals (Table).

Cerebral and cardiac perfusion. Despite induction of profound hypothermia and circulatory arrest at a brain temperature of 10°C, no decrease in cerebral oximetry values was observed (Fig. 3, *b*).

During the 12 hours of rewarming, cerebral oximetry values remained consistently above the threshold of 40%, indicating adequate cerebral perfusion. Later, however, with progressive hypotension poorly controlled by vasopressors, oximetry values dropped to 20% and below. At the same time, intracranial pressure (ICP) remained within acceptable limits in 4 out of 5 animals. In animal No. 3, ICP increased after 16 hours of the experiment, followed by death (Table).

Systemic coagulopathy and ischemia-reperfusion injury. When the T_n reached 26–30°C, 3 of the 5 animals developed severe coagulopathy char-

Table. Summary of data on experimental animals, treatment outcomes, and causes of death.

Parameter	The values of the parameters for each individual olive baboon (*Papio anubis*)				
	1	**2**	**3**	**4**	**5**
Sex	male	male	male	male	male
Body weight, kg	19.8	19.6	18.0	21.0	26.7
Blood loss, mL	820	500	300	700	600
Time to cooling to 10°C, min	35	7	37	23	11
ROSC	—	+	+	+	+
ECMO weaning	—	+	—	—	+
Extubation	—	+	—	—	+
Survival time from the start of the experiment, hours	15	44	16	19	19
Main pathological findings	Pulmonary edema, multiple organ failure	Myocardial infarction	Pulmonary edema, cerebral edema	Multiple organ failure, small intestine ischemia, abdominal compartment syndrome	Pulmonary edema

Note. ROSC — return of spontaneous circulation; ECMO — extracorporeal membrane oxygenation.

acterized by bleeding from surgical access sites and diffuse intra-abdominal hemorrhage. These complications required surgical wound revision, use of electrocautery, application of local hemostatic agents, tight wound packing, and intravenous administration of 1.0 g tranexamic acid. Moderate acceleration of the rewarming process at these temperatures was found to help bypass the coagulopathy phase more quickly, reducing the risk of fatal blood loss.

In one animal (No. 2), efforts to control bleeding from a wound in the right axillary region resulted in inadvertent decannulation, causing massive hemorrhage. The bleeding was promptly controlled by clamping the vessel and ligating the axillary artery. Mechanical circulatory support was maintained exclusively through the femoral arterial cannula, with no evidence of upper limb ischemia. The animal was later successfully extubated.

During the first 5–6 hours of rewarming, lactate levels remained elevated in all animals (approximately 10 mmol/L), but could be managed by increasing the perfusion index, transfusing blood, and, if necessary, removing excess fluid from the circulating blood. However, despite intensive therapy, lactate levels subsequently rose rapidly to the maximum measurable by the device (15 mmol/L). In animals that later succumbed, lactate concentrations exceeded 15 mmol/L from the 8th hour of rewarming. In contrast, surviving animals showed successful control of progressive lactic acidosis. Systemic reperfusion was identified as the most significant factor contributing to mortality (Fig. 3, *b*).

Blood biochemistry showed an increase in transaminase activity: AST increased from 55.4 (46.8–64.0) to 171.4 (150.4–192.3) and then to 301.3 (239.9–362.6) U/L, while ALT increased from 14.5 (5.8–23.1) to 20.9 (17.8–24.0) and then to 48.3 (16.3–80.3) U/L at 5 and 7 hours after the start of rewarming. In addition, creatinine concentrations increased from 149 (145–152) to 155 (115–196) and then to 190 (135–245) μmol/L at 7 and 9 hours, respectively, reflecting systemic hypoperfusion and the development of multiorgan failure. However, these parameters were successfully corrected in surviving animals by intensive therapeutic measures (Fig. 4, *a*, *b*, *c*).

A significant increase in creatine phosphokinase (CPK) activity was also observed, rising from 202 (200–205) to 1105 (1042–1167) and subsequently to 2045 (1710–2380) U/L at 5 and 7 hours after rewarming, respectively (Fig. 4, *d*). In animals that did not survive, CPK levels remained elevated, whereas in survivors, these levels returned closer to baseline levels.

Survival. Endotracheal extubation was successful in 2 of 5 animals. Their cooling times were 7 and 11 minutes, respectively. One animal underwent extubation after cannula removal 16 hours after the start of the experiment. Although it was returned to the vivarium, the animal died 44 hours into the experiment without regaining full consciousness. This precluded assessment of brain function. Autopsy revealed ischemic myocardial damage and moderate cerebral edema.

Another animal survived the procedure with successful tracheal extubation. However, while being moved to the vivarium, it developed respiratory failure, probably due to respiratory muscle fatigue, and subsequently died. Postmortem examination revealed severe pulmonary edema (Fig. 5).

Both «surviving» animals showed purposeful motor activity, including fist clenching, spontaneous eye opening, and mouth movements.

Three of the five animals died on the operating table 14.5 to 18.5 hours after the start of rewarming. It took 23, 35, and 37 minutes, respectively, to reach the target T_n. According to postmortem findings, the primary causes of death were pulmonary edema and, in some cases, ischemic liver injury.

Animal No.4 developed abdominal compartment syndrome (ACS) due to excessive fluid therapy, requiring

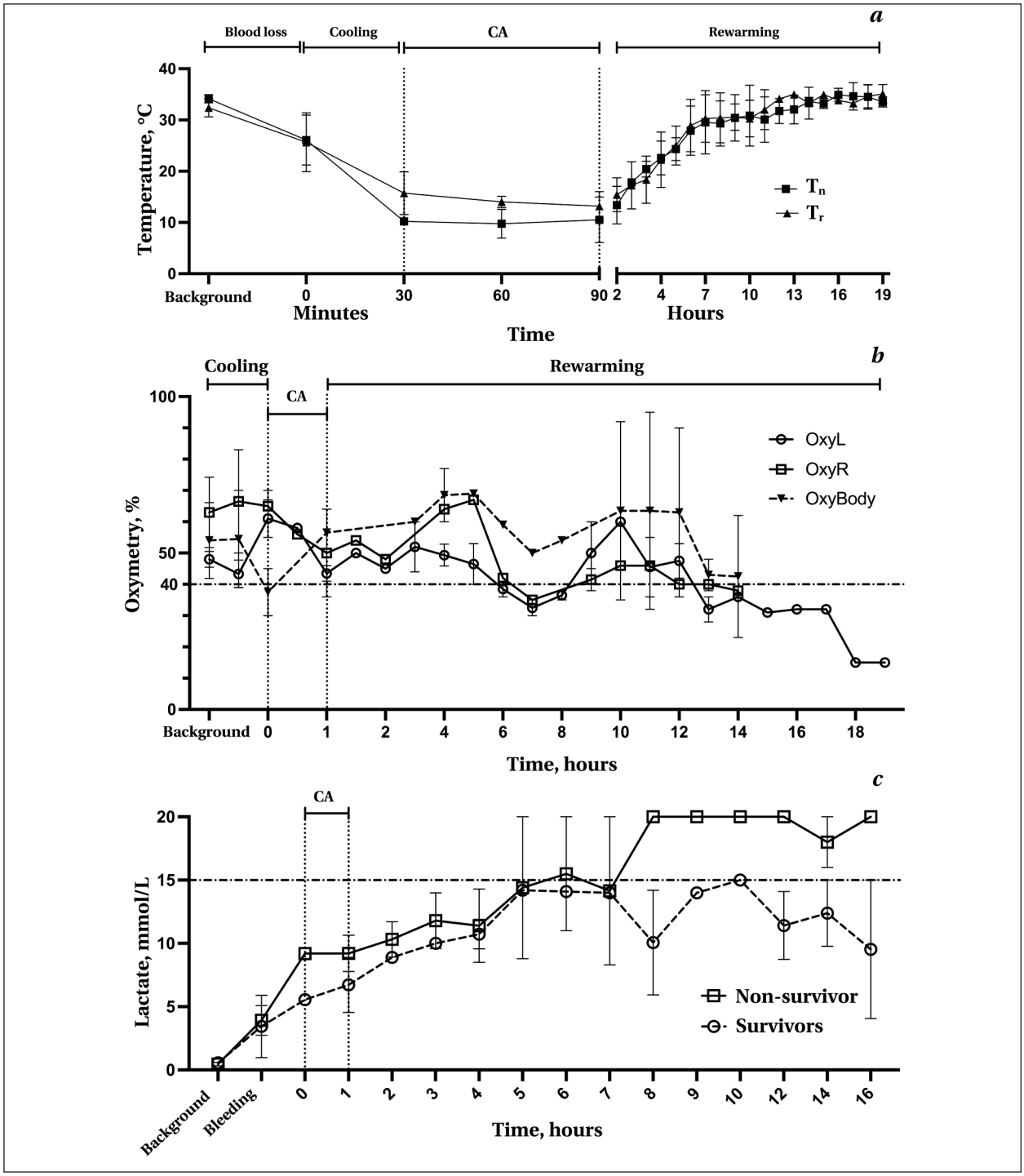

Fig. 3. Changes in key parameters monitored during the experiment.
Note. *a* — nasopharyngeal (Tn) and rectal (Tr) temperatures; *b* — cerebral oximetry in the left and right hemispheres (OxyL and OxyR) and tissue oximetry in the lumbar region (OxyBody); *c* — serum lactate concentrations, stratified by surviving (*N*=2) and non-surviving (*N*=3) animals. The horizontal line at 15 mmol/L indicates the threshold measurable by the blood gas analyzer. Values above this line, shown at the 20 mmol/L level, are presented for illustrative purposes and were excluded from the calculations. CA — circulatory arrest.

a relaparotomy and laparostomy (Bogotá bag). This animal also had progressively increasing ICP during rewarming, reaching 47 mmHg, which was refractory to conservative management and remained elevated after resolution of the ACS. Postmortem examination revealed cerebral edema in addition to total ischemic damage to the small intestine.

Histologic examination. All animals showed signs of cerebral edema of varying severity, dystrophic changes in neurons (cytoplasmic vacuolization, nuclear deformation), and plasmatic imbibition of vascular walls (Fig. 6, *a*). Myocardial changes were also observed in all cases, ranging from diapedetic microhemorrhages, edema, and fragmentation of

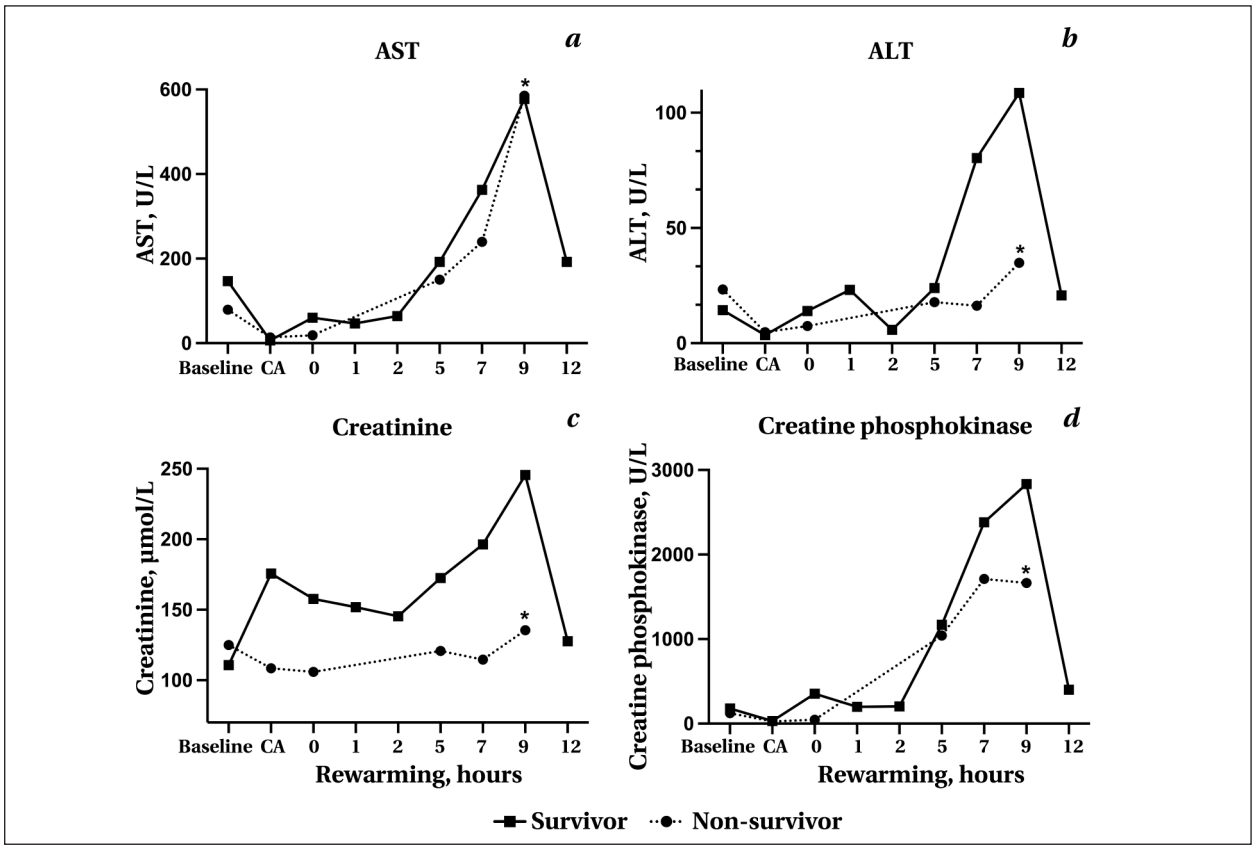

Fig. 4. Trends in key biochemical parameters in representative animals: one surviving and one non-surviving.
Note. *a* — AST; *b* — ALT; *c* — creatinine; *d* — creatine phosphokinase. * — final time point of biochemical analysis for the non-survivor; CA — circulatory arrest.

individual muscle fibers to intramural necrosis. Pulmonary findings included acute alveolar emphysema, focal dystelectasis, congestion, and inflammatory infiltration.

In the animal that died 44 hours into the experiment, cerebral edema was accompanied by extensive non-coronary myocardial necrosis of the left ventricle and alveolar pulmonary edema (Fig. 6, *b*). The animal with ACS demonstrated necrosis of the intestinal wall and hydropic degeneration of hepatocytes (Fig. 6, *c*). Renal histopathology was mild and included exudative glomerulopathy, tubular and interstitial edema, and tubular epithelial degeneration (Fig. 6, *d*).

Thus, this study demonstrated the feasibility of using the EPH technique in TCA. Target brain (10°C) and body (16°C) temperatures were successfully achieved in all animals within a short time, ensuring adequate cerebral oxygenation, allowing for surgical intervention and further controlled rewarming of the body. Return of spontaneous circulation was achieved in 4 out of 5 animals, and 2 animals were successfully weaned from ECMO and extubated. However, both animals died in the early postoperative period, precluding evaluation of cognitive function. The main causes of mortality during the rewarming period were ischemia-reperfusion injury, cerebral edema and transient coagulopathy.

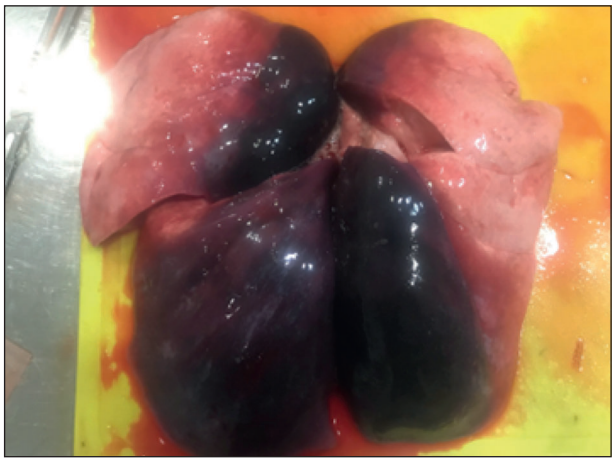

Fig. 5. Postmortem photograph of the lungs of one of the animals (No. 5) that died after tracheal extubation due to progressive respiratory failure and development of acute respiratory distress syndrome (ARDS).

Prolonged blood collection leading to systemic hypoxemia and hypoperfusion, the inability to achieve rapid cooling in all cases, and a period of no-flow in the vessels of the lower half of the body during cooling of the upper half (T_n reached 10°C while T_r remained at 35°C) resulted in severe systemic ischemia. This ischemia caused an accumulation

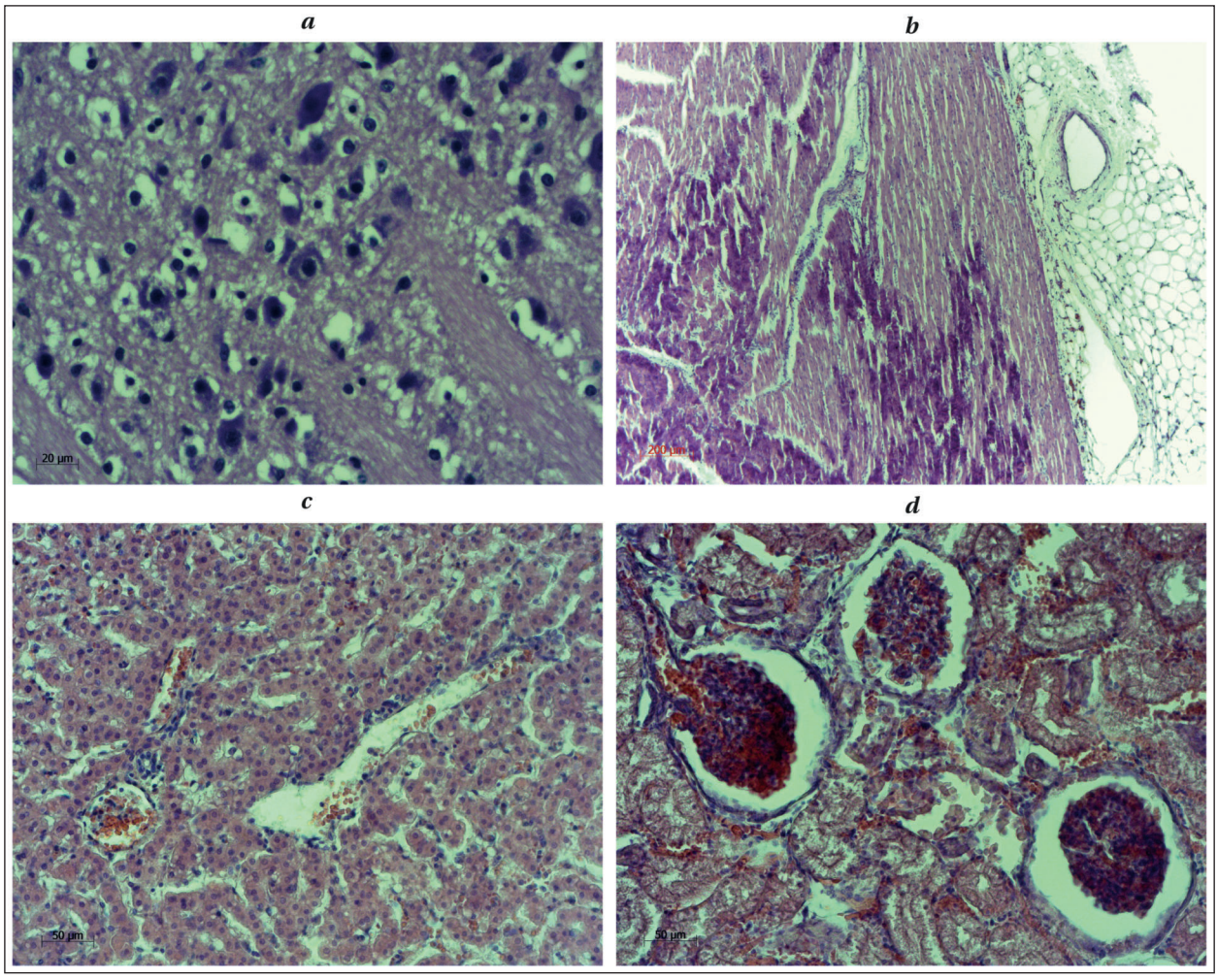

Fig. 6. Representative microstructural images of vital organs from animals subjected to emergency profound (ultra-deep) hypothermia.
Note. a — Thalamus: cerebral edema, plasmatic imbibition of vascular walls, dystrophic neuronal changes (cytoplasmic vacuolization, nuclear deformation) (hematoxylin-eosin stain, ×400). *b* — Lateral wall of left ventricular myocardium: intramural myocardial necrosis (dark purple staining, hematoxylin-eosin, ×50). *c* — Liver: perisinusoidal edema, severe hydropic degeneration of hepatocytes (hematoxylin-eosin, ×200). *d* — Kidney: exudative glomerulopathy, tubular and interstitial edema, dilatation of glomerular capsules, proliferation of parietal epithelial cells and hydropic degeneration of tubular epithelium (hematoxylin-eosin, ×200).

of under-oxidized metabolites in the internal organs and muscles of the lower extremities. Subsequently, despite lowering the T_r to target levels, rewarming was complicated by rhabdomyolysis (confirmed by a significant increase in creatine phosphokinase activity) and severe reperfusion injury, which could not be alleviated by slow temperature rise.

Studies by others have shown that to prevent severe reperfusion injury, cooling must be as rapid as possible while rewarming must be slow. An optimal cooling rate of 2°C/min was demonstrated by H. Alam et al. in porcine models [14]. This was achieved by open cannulation of the aorta and right atrium with large diameter cannulae after resuscitative thoracotomy. However, too rapid cooling carries the risk of cold-induced damage to vital organs, while too slow cooling increases the likelihood of ischemia-reperfusion syndrome.

Therefore, T_n should ideally be reduced to 26°C within approximately 13 minutes. This was achieved only twice in the current study, specifically in the two animals classified as «survivors» (7–11 minutes). However, in the animal that survived 44 hours after the procedure and reached the target T_n in 7 minutes, postmortem examination revealed a massive myocardial infarction, which could be partially attributed to cold-induced injury.

The progression of ischemia-reperfusion syndrome is evidenced by a gradual increase in serum lactate concentration (> 15 mmol/L), likely due to a combination of factors: prolonged exsanguination, one minute of normothermic circulatory arrest, three minutes of chest compressions (generally ineffective in the setting of massive blood loss), prolonged lower body ischemia during upper body cooling, and 60 minutes of hypothermic circulatory

arrest. Notably, as several studies have shown, hypothermic circulatory arrest of this duration is not inherently a cause of irreversible cellular or organelle damage, as evidenced by survival rates exceeding 50% with favorable functional outcomes [17, 18].

The observed uncontrolled rise in lactate concentration during the early phases of reperfusion likely serves as a prognostic marker for adverse outcomes [19]. Similar lactate levels were recorded in a previous experiment using EPH during simulated tactical military medical exercises [20]. Large pigs with abdominal gunshot wounds underwent EPH and were surgically treated in a reinforced concrete bunker simulating a forward surgical care environment. They were transported 50 km in a hypothermic state, without mechanical ventilation or circulatory support, to a simulated advanced surgical care facility where rewarming was initiated. It took over an hour to reach the target T_n. Although ROSC was achieved after two hours of transport, the animals ultimately succumbed to severe reperfusion injury [20].

Another critical issue affecting survival was cerebral edema, which was observed in all animals. This was primarily related to inadequate replacement of blood loss with crystalloids and colloids. Despite monitoring of ICP and cerebral oximetry, as well as pharmacologic and non-pharmacologic intensive care, it was not possible to completely resolve this complication. The death of the only long-lived animal was primarily due to progressive cerebral edema, as confirmed by autopsy findings. During the experiment, computed tomography imaging was not available to assess brain damage.

Finally, one of the life-threatening problems was coagulopathy, which consistently manifested itself when temperatures reached 27–32°C during rewarming. This was characterized by diffuse bleeding from existing wounds and access sites (including puncture sites), requiring both surgical and pharmacologic hemostasis. As a result, the volumetric flow in the ECMO circuit decreased, requiring additional fluid infusion and subsequently provoking ischemia-reperfusion. Intravenous administration of tranexamic acid was found to have little effect on the intensity of bleeding. S. Tisherman and colleagues also highlighted the challenges of managing coagulopathic bleeding during the rewarming phase and addressed this by increasing the rewarming rate until bleeding stopped [15].

Therefore, slow rewarming is necessary to avoid severe reperfusion injury and edema; however, too slow rewarming results in coagulopathic bleeding, which can be fatal with extensive tissue damage. Similarly, cooling must be as rapid as possible, but too rapid cooling can cause cold injury to vital organs.The optimal cooling and rewarming rates require further investigation. Our experimental data indicate that the best results are achieved with cooling over 10–15 minutes and rewarming over at least 12–16 hours.

Encouraging results from numerous U.S. research groups demonstrating sufficient animal survival after two hours of circulatory arrest with EPH led the FDA to approve a pilot randomized controlled clinical trial (EPR-CAT) enrolling 10 patients in an EPH group and 10 in a control group. Due to the COVID-19 pandemic, the trial was temporarily suspended and the results remain unpublished. Furthermore, to our knowledge (personal communication with S. Tisherman), there have been no successful human cases. Nevertheless, the FDA's approval of this trial highlights the significant interest in hypothermia for terminal conditions and novel approaches to saving civilian and military casualties.

Our study has several limitations that may have significantly influenced the results. First, the EPH procedure is resource-intensive, requiring high-quality (around-the-clock) monitoring, intensive care, and extensive use of blood and blood products, which is difficult to achieve in an experimental operating room. At a minimum, animals should have undergone computed tomography (CT) or magnetic resonance imaging (MRI) of the brain, electroencephalography, and coagulation studies after extubation; these were not available at the time. While blood and blood product replacement is preferable to crystalloid solutions for volume resuscitation, donor blood components are rarely available in animal studies and virtually never in nonhuman primate studies.

Second, the cooling rate was relatively slow for both the upper and lower body. Achieving target temperatures more rapidly would have required larger diameter cannulas than the 15–17 Fr cannulas we used. In animals weighing approximately 20 kg, larger cannulas would require open aortic and vena cava cannulation, which carries additional risks of surgical trauma and bleeding.

Finally, the small number of animals and the lack of a control group are significant limitations due to the pilot nature of this study.

Nevertheless, the results of this first-of-its-kind experiment in non-human primates underscore the importance of pursuing novel solutions to help those previously considered beyond saving. The concept of using EPH, developed by our esteemed compatriot V. A. Negovsky and his successors, including P. Safar and his student S. Tisherman, is both technically and physiologically feasible in the context of terminal states. Rapid cooling of the brain, heart, and, if necessary, the entire organism during TCA allows a 1–2-hour window for life-saving surgery, blood product procurement, and transport to a specialized center where gradual rewarming can restore vital functions. Demonstrating the effi-

cacy of this method will open new avenues for exploring various applications of rapid whole-body external cooling, allowing temporary preservation not only of organs and tissues, but of the entire organism, followed by rewarming after life-threatening injuries have been treated.

Conclusion

The experiment showed that emergency profound (ultra-deep) hypothermia, with head cooling to 10°C and core cooling to 16°C, preserves adequate perfusion of vital organs and tissues despite 60 minutes of complete circulatory arrest. In addition, faster cooling rates are associated with better chances of recovery and survival. During rewarming, cardiac function is restored and successful management of ischemia-reperfusion syndrome, vital organ edema and coagulopathy leads to resuscitation. Further research, including studies in other animal models, is needed to better understand the causes of serious complications and to refine the EPH protocol.

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