https://doi.org/10.15360/1813-9779-2024-2-55-64

Asphyxial Circulatory Arrest with a Complex of Resuscitation Measures in an Experimental Model

Alexey Y. Dubensky[1,2], Ivan A. Ryzhkov[3]*, Konstantin N. Lapin[3], Sergey N. Kalabushev[3], Lydia A. Varnakova[3], Zoya I. Tsokolaeva[3], Vladimir T. Dolgikh[3], Andrey V. Grechko[4]

[1] Institute of Higher and Additional Professional Education,
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[2] N. I. Pirogov National Medical Surgical Center, Ministry of Health of Russia,
70 Nizhnyaya Pervomayskaya Str., 105203 Moscow, Russia
[3] V. A. Negovsky Research Institute of General Reanimatology,
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia
[4] Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia

For citation: *Alexey Y. Dubensky, Ivan A. Ryzhkov, Konstantin N. Lapin, Sergey N. Kalabushev, Lydia A. Varnakova, Zoya I. Tsokolaeva, Vladimir T. Dolgikh, Andrey V. Grechko.* Asphyxial Circulatory Arrest with a Complex of Resuscitation Measures in an Experimental Model. *Obshchaya Reanimatologiya = General Reanimatology.* 2024; 20 (2): 55–64. https://doi.org/10.15360/1813-9779-2024-2-55-64 [In Russ. and Engl.]

***Correspondence to:** Ivan A. Ryzhkov, riamed21@gmail.com; Alexey Y. Dubensky, dubkoal@gmail.com

Summary

The majority of asphyxial circulatory arrest (CA) models have a number of disadvantages, such as the lack of uniform criteria for fixing CA and recovery of spontaneous circulation, short duration of CA episode and limited volume of post-resuscitation intensive care, poor similarity with resuscitation measures in current clinical anesthesiology/intensive care settings.

The aim of the study: to improve the experimental model of asphyxial CA by standardizing experimental procedures and using a complex of resuscitation measures replicating current CA management in clinical anesthesiology-intensive care.

Materials and methods. The experiments were conducted on 34 male Wistar rats, distributed into 2 groups: Group I included animals subjected to sham procedure (SP, *N*=12) and Group II — animals subjected to asphyxial circulatory arrest (CA, *N*=22) and subsequent resuscitation. Asphyxia in anesthetized rats was induced by rocuronium bromide injection, followed by recording of electrocardiogram (ECG), parameters of invasive blood pressure (BP) measurement and laser Doppler flowmetry (LDF) to assess skin perfusion. CA episode was maintained for 2 min, followed by a series of resuscitation measures and intensive therapy for 2 h. Circulatory parameters (ECG, BP, LDF), gas composition and arterial blood acid-base state (ABS) dynamics were evaluated.

Results. Monitored parameters were comparable in both groups at baseline after stabilization period. After exclusion criteria were applied 11 animals from SP group and 18 — from CA were included in the analysis. Tachycardia (heart rate, beats/min^{-1}, SP vs CA) was documented in the CA group: 218 [205; 236] vs 286 [272; 305], $P \leqslant 0.0001$), as well as recovery of skin perfusion to subnormal parameters in the first minutes after successful resuscitation. At minute 10 in the post-resuscitation period worsening of skin perfusion (M, perfusion units, SP vs CA): 14.7 [12.1; 16.5] vs 10.1 [7.0; 12.5], $P=0.0014$), and decompensated mixed acidosis (pH, SP vs CA): 7.42 [7.40; 7.43] vs 7.20 [7.13; 7.23], $P \leqslant 0.0001$) were documented in the CA group, however BP values were comparable (BP, mmHg, SP vs CA): 60 [58; 72] vs 67 [62; 82], $P=0.482$). At minute 120 post-resuscitation and at the end of intensive care period, both groups demonstrated similar values of the monitored parameters. Three out of 18 animals in the CA group died after resuscitation.

Conclusion. Electromechanical dissociation underlies CA in rats subjected to asphyxia. The use of LDF to assess peripheral blood flow makes it possible to standardize the severity of ischemic reperfusion injuries and improve reproducibility of the model. Series of resuscitation measures in experimental setting is justified from a bioethical point of view, and makes it possible to improve repeatability of preclinical research results in clinical practice.

Keywords: circulatory arrest; asphyxia; resuscitation measures; experimental model; rat

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

Introduction

Cardiac arrest (CA) is a critical condition in which there is no effective circulation. It is generally divided into out-of-hospital and in-hospital cardiac arrest. According to the European Resuscitation Council, the incidence of out-of-hospital CA in Europe is 67–170 cases per 100,000 population per year, and the survival rate of these cases (at hospital discharge) is approximately 8% [1]. The proportion of out-of-hospital CA in the total number of cardiac arrests is between 45% and 55% [2]. In-hospital cardiac arrest can occur in any hospitalized patient.

According to L. W. Andersen et al, the number of cases of in-hospital CA in the USA reaches 290,000 per year. The most common causes of CA are cardiac (50–60%), while the incidence of CA due to respiratory failure varies from 15% to 40% [3].

Currently, despite the development of new medical technologies, improvements in resuscitation protocols, and supportive therapy in the post-resuscitation period, the disability and mortality from sudden cardiac arrest remain dismal [4] and have not changed much over the past decade (10.4%) [5]. Thus, according to Patel et al, the outcome of in-hospital CA is highly variable depending on the medical care provided, with in-hospital survival of CA ranging from 45% to 85% and one-year survival varying from 5% to 35% [6].

Based on etiology, CA is classified as primary, which is due to cardiac causes, and secondary, which develops because of extracardiac factors [7]. In most cases, primary CA is caused by ventricular fibrillation (VF) or pulseless ventricular tachycardia (VT), for which electrical cardiac defibrillation is effective [8].

Secondary CA is most commonly caused by progressive respiratory and circulatory hypoxia, severe metabolic derangements (acidosis, hypo- and hyperkalemia), hypothermia, tension pneumothorax, cardiac tamponade, and intoxication, all of which are considered «irreversible» and require emergency treatment [9]. Asphyxial CA develops due to impaired gas exchange resulting in severe hypoxemia, hypercapnia, and tissue hypoxia. Respiratory acidosis and hypoxia damage the sinoatrial node and cardiac conduction system, resulting in bradyarrhythmias. If asphyxia persists and gas exchange is impaired, bradycardia progresses to electromechanical dissociation (EMD) or asystole [10, 11].

Experimental models of asphyxial CA in laboratory animals more closely resemble the mechanisms of CA in pediatric practice and in patients with acute respiratory failure of various etiologies [12]. Katz et al. developed one of the first such models in 1995 [13]. Disconnection of a halothane anesthetized experimental animal from the ventilator under neuromuscular blockade induced asphyxial CA. CA was diagnosed when the blood pressure dropped below 10 mmHg. Resuscitation procedures reflected current clinical approaches at the time. This model was later updated with different anesthetics, durations of asphyxia, and changes in the range of resuscitation procedures [14, 15].

There are several common drawbacks to the experimental models of asphyxial CA described here. The lack of a standardized approach to anesthetic selection may have an indirect effect on macro- and microhemodynamic parameters, leading to biased data [16]. The duration of CA varies depending on the individual characteristics of the experimental animal and this has a direct effect on the success of resuscitation. For example, if CA lasts 7 minutes, the chance of restoring spontaneous circulation is less than 50% [17]. Other drawbacks include the lack of uniform and clear criteria for recording arrest and return of spontaneous circulation, the short duration (30–120 minutes) and limited volume of intensive care after resuscitation, and the low consistency with actual resuscitation practice in current clinical anesthesiology and intensive care.

The aim of this study was to improve the experimental model of asphyxial CA by standardizing the experimental procedures and applying a set of resuscitation measures similar to those used in patients with CA in current clinical anesthesiology and intensive care.

Materials and Methods

A prospective randomized controlled experimental study (in vivo) was conducted at the V. A. Negovsky Research Institute of General Reanimatology, Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology (FRCCICMR), Moscow, Russia, on 34 adult male Wistar rats weighing 250–350 g. The animals were divided into two groups: group 1, sham-operated animals (SO group, *N*=12); and group 2, animals with asphyxial CA and subsequent resuscitation (CA group, *N*=22). Animals were deprived of food but had free access to water for 12 h prior to the experiment.

The study was conducted in accordance with accepted national and international bioethical standards (Directive 2010/63/EU). The study protocol was approved by the Local Ethics Committee of the FRCCICMR (Protocol No. 4/21/7, dated September 29, 2021).

The following exclusion criteria were used: serious complications or death during the experiment before induction of cardiac arrest (side effects of anesthesia, complications of the surgical manipulations performed) and achievement of a humane endpoint of the study (severe trauma, pain and suffering of the animal that cannot be alleviated by the available means).

Anesthesia and surgical manipulations. All animals included in the experiment were anesthetized with a combination of tiletamine/zolazepam («Zoletil 100», Virbac) 20 mg/kg + xylazine («Xylanit», LLC «NITA-FARM», Russia) 5 mg/kg intraperitoneally with additional administration of Zoletil 10 mg/kg at the first signs of animal arousal.

The left carotid artery and the left internal jugular vein were catheterized with a PE-50 polyethylene catheter (OD 0.95 mm, ID 0.58 mm, SciCat, Russia) for the purpose of invasive blood pressure (BP) measurement and arterial blood sampling, drug administration, and implementation of

the postresuscitation intensive care protocol, according to the previously described method [18]. If necessary, the catheter was flushed with 0.1–0.2 ml of unfractionated heparin solution (20 U/mL) to maintain patency.

Tracheal intubation and ventilation. To ensure adequate ventilation during preparation and resuscitation, the trachea was intubated via direct laryngoscopy using a 16G venous catheter. After neuromuscular blockade by intravenous administration of rocuronium bromide 1.4 mg/kg body weight, ventilation was performed with the SAR-1000 (CWE Inc., USA) in CMV/VC mode. The tidal volume (V_t) was calculated according to the nomogram in the ventilator manual (approximately 0.7 ml per 100 g body weight), with an oxygen fraction (FiO_2) of 21%, a respiratory rate (f) of 60/min, and an inspiration/expiration ratio (I:E) of 1:2. If spontaneous breathing persisted, a repeated dose of rocuronium bromide was administered.

Preparatory measures. Rats were restrained in the supine position on a heated MouseMonitor S monitor platform (INDUS Instruments, USA) (Fig. 1). A rectal thermometer was used to measure and control central body temperature. The target central body temperature was 36.0–37.0°C. To prevent heat loss, the animal was covered with insulating material. The animals were allowed to stabilize for 15–20 min before measurements were started.

BP measurement. The arterial catheter was connected to a Deltran DPT-100 transducer (Utah Medical Products, USA) via a Y-piece and infusion line. The analog BP signal from the transducer and BP-100 device (CWE Inc., USA) was transmitted to a PowerLab16/35 device (ADInstruments, Australia) connected to a personal computer (PC). The digitized BP signal was recorded, stored in the PC's hard disk memory, and analyzed using LabChart Pro 8 software. The mean arterial pressure (BPmean) for the measurement period (5 min) was calculated from the BP curve data.

ECG registration. The analog ECG signal from the surface electrodes of the MouseMonitor S platform (INDUS Instruments, USA) was transmitted to a PowerLab16/35 device (ADInstruments, Australia) connected to a PC. The digitized ECG signal in three standard leads (I, II, III) was recorded, stored in the PC hard disk memory and analyzed using LabChart Pro 8 software. The average heart rate (HR) for the measurement period (5 min) was calculated from the ECG data.

Arterial blood gas and acid-base balance (ABB). Arterial blood (0.2 ml) was withdrawn from the arterial catheter into an «insulin» syringe (1.0 ml) after prior aspiration of the residual flushing solution from the catheter. The syringe walls were pretreated with unfractionated heparin 5,000 U/mL (no more than 50 µL). Arterial blood gases and ABB (pH, pCO_2, pO_2, BE, HCO_3, SaO_2, and lactate levels) were analyzed using CG4+ reagent cartridges for the iSTAT 1 analyzer (Abbott Point of Care Inc., USA).

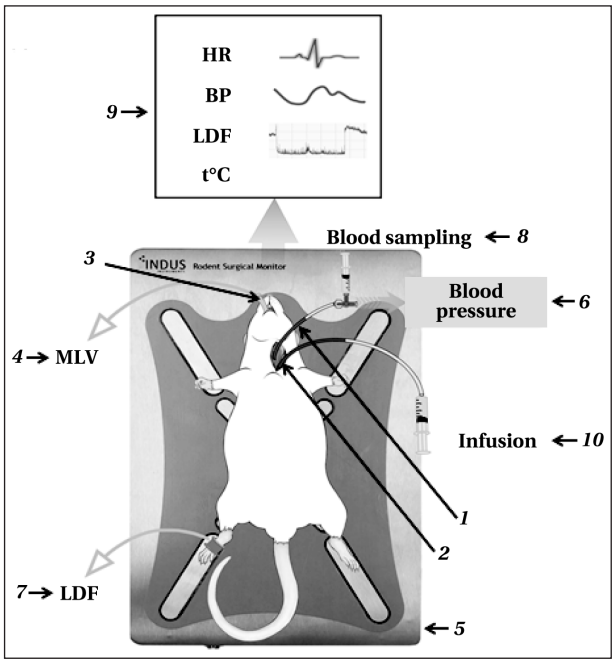

Fig. 1. Schematic of the asphyxial cardiac arrest modeling experiment.
Note. *1* — arterial catheter; *2* — venous catheter; *3* — intubation tube; *4* — MLV (mechanical lung ventilation) using ventilator for small laboratory animals; *5* — heated platform of the monitor of functional parameters of small laboratory animals; *6* — device for direct measurement of blood pressure; *7* — LDF (laser Doppler flowmetry) device; *8* — sample of arterial blood for assessment of blood gases and acid-base status; *9* — graphic representation of recorded physiological parameters (ECG, blood pressure, body temperature, LDF); *10* — syringe-doser for continuous intravenous infusion in the post-resuscitation period.

Registration of skin perfusion by laser Doppler flowmetry (LDF). The right hind paw of the rat was wiped with a wet gauze cloth to clean the skin surface. The optical probe of the LAZMA MC-3 device (LLC SPE «LAZMA», Russia) was placed perpendicular to the central part of the plantar surface of the animal's foot. To fix the probe tip to the skin surface, a strip of soft adhesive plaster was wrapped around the tip and the paw of the animal. LDF registration took 5 minutes. LDF software version 3.2.0.475 (OOO NPP «LAZMA», Russia) was used to calculate the mean perfusion value *(M)* measured in conventional perfusion units (pfu).

Model of asphyxial cardiac arrest and resuscitation. After registration of baseline parameters (Fig. 2), we modeled CA according to a previously described method [19] with the author's modifications. The animal was reinjected with myorelaxant (rocuronium bromide 1.4 mg/kg) and the breathing circuit of the ventilator was disconnected (Fig. 3). ECG, BP, and LDF monitoring was continued to determine the time of CA. When mean BP (BP_{mean}) decreased to 20 mmHg in combination with marked bradycardia and skin perfusion decreased to the

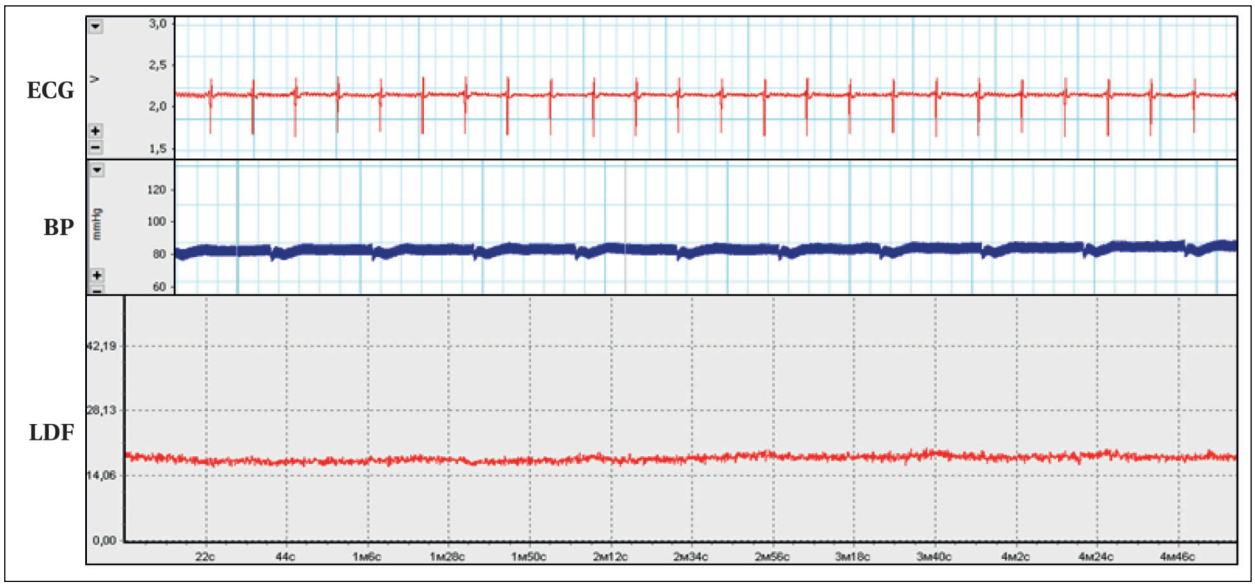

Fig. 2. Recorded blood pressure (BP), electrocardiogram (ECG), and laser Doppler flowmetry (LDF) signals in the anesthetized rat at baseline (before induction of asphyxia).

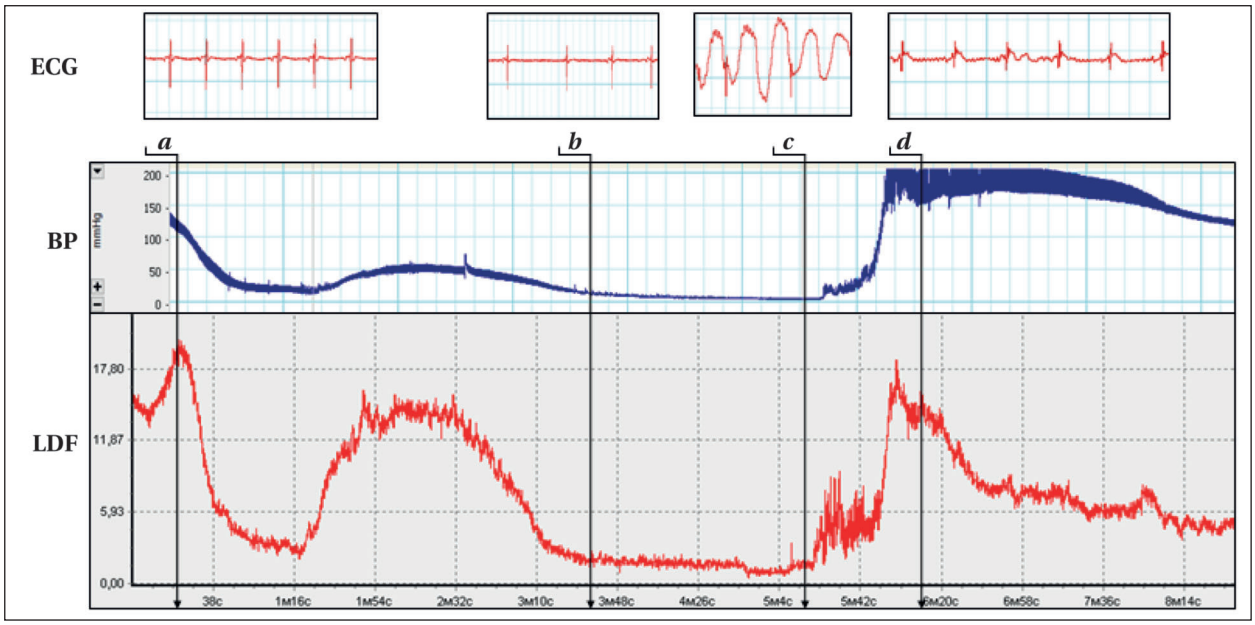

Fig. 3. Changes in ECG, BP and LDF parameters during induction of asphyxia, cardiac arrest and resuscitation.
Note. *a* — induction of asphyxia; *b* — onset of cardiac arrest; *c* — onset of resuscitation; *d* — return of spontaneous circulation. In the above example, at the 2nd minute of asphyxia, a transient increase in blood pressure and skin perfusion was recorded in the anesthetized animal, preceded by a marked decrease (agonal period).

level of LDF equal to «biological zero» (3–4 pfu), it was considered that there was no effective tissue perfusion, and the onset of cardiac arrest was recorded (Fig. 3). Resuscitation was initiated 2 minutes after CA (Fig. 3). Mechanical ventilation was resumed in CMV/VC mode with the following parameters: FiO_2=100%, f=80/min, I:E=1:2, and V_t according to the rat nomogram. Chest compressions were performed in an anteroposterior direction in the supine position at a rate of 200/min, the depth of compression was 1/3 of the anteroposterior size of the chest, followed by complete decompression of the chest. Adrenaline 0.005 mg/kg was then administered intravenously.

After one minute of chest compressions, chest compressions were stopped and heart rate, mean blood pressure, and skin perfusion were assessed. If CA persisted, resuscitation was continued with cardiac rhythm assessment every minute. Repeated administration of 0.005 mg/kg epinephrine was performed every 5 minutes of resuscitation. ECG, BP, and LDF monitoring continued. If resuscitation was ineffective within 10 minutes, it was stopped. When spontaneous circulation was restored (increase

in BP_{mean} above 50 mmHg, increase in skin perfusion above the «biological zero» of LDF (Fig. 3)), lung ventilation with oxygen was continued along with monitoring of BP, ECG, LDF, infusion of NaCl 0.9% solution 10 mL/kg/h.

Blood gases and ABB were measured 5 minutes after resuscitation and the results were used to adjust the ventilatory parameters. In cases of severe metabolic acidosis (pH <7.2, BE <–10 mmol/L), infusion of 4% $NaHCO_3$ 1 mmol/kg was performed. Two hours after resuscitation (after completion of measurements), a spontaneous breathing test was performed, the breathing circuit was disconnected from the endotracheal tube, and spontaneous breathing attempts were recorded for 2 minutes.

Study time points. The parameters studied were recorded at baseline after the stabilization period (time point 1), at 5–10 min (time point 2), and at 115–120 min (time point 3) after return of spontaneous circulation. Animals in the SO group underwent the same measurements and procedures as those in the CA group, except for induction of asphyxia, CA, and resuscitation (chest compressions, administration of adrenaline, ventilation with FiO_2 of 100%).

Data processing and statistical analysis. The parameters measured during the experiment were recorded in the experimental flow chart (body weight, body temperature, arterial blood ABS) and in the PC hard disk memory (ECG, BP, LDF) using the appropriate software. At the end of the experiment, the primary data were analyzed and the studied parameters were calculated. The sample size was calculated in the StatMate 2.0 program (GraphPad Software, USA) based on the previous series of experiments on rat skin microcirculation, taking into account the variability of skin perfusion indices (according to LDF data), the estimated mortality in the CA group of about 30%, and the power of the method of more than 0.9. The M value used in the sample calculation was 16.22 pfU, and the standard deviation was 2.22 pfU. The results were analyzed using the software packages Statistica 13.0 (StatSoft, USA) and Prism 8 (GraphPad Software, USA). Because most of the parameters studied had a non-normal distribution (based on the Shapiro–Wilk test), the Mann–Whitney *U*-test was used to assess the significance of differences between groups, and the Friedman test was used to assess the change in the index within group (for pairwise comparisons, the Wilcoxon test with Bonferroni correction was used). Results are presented as median and interquartile range *Me* [25%; 75%]. Differences were considered significant when $P<0.05$.

Results and Discussion

According to the exclusion criteria, one animal was excluded from the SO group (N=12) and four from the CA group (N=22). Thus, 11 animals from the SO group and 18 from the CA group were used in the subsequent analysis. There were no deaths in the SO group, whereas in the CA group, 3 out of 18 animals (16.7%) died after resuscitation.

In the CA group, the time from induction of asphyxia to the moment when mean blood pressure fell below 20 mmHg in combination with marked bradycardia and reduction of skin perfusion to the level of LDF «biological zero» (3–4 pfU) was evaluated (CA documentation). The mean time from induction of asphyxia to CA documentation was 220 seconds [180; 255], and the total time from induction of asphyxia to return of spontaneous circulation was 330 seconds [300; 375] in the CA group. After induction of asphyxia, one third of the animals in the CA group showed a transient decrease in BP below 20 mm Hg, a decrease in M according to LDF to the level of «biological zero» with a gradual return to subnormal values, and no pathological rhythms on the ECG. These episodes were considered the agonal period, and the time between the induction of asphyxia and the documentation of AC was prolonged.

During the first minutes of return of spontaneous circulation in the CA group, the following changes in the studied functional parameters were observed: arterial hypertension up to 200/160 mmHg, increase in M to subnormal values, and sinus tachycardia. At baseline, there was no difference between the groups in any of the parameters studied (Table). Ten minutes after resuscitation and 2 hours after return of spontaneous circulation, there was no statistical difference in BP_{mean} between groups. Ten minutes after resuscitation, the CA group had a significantly higher HR values than the SO group (Table). The CA group had a lower mean skin perfusion (M) at the 10th minute of the postresuscitation period, but there was no difference between the groups at the 120th minute (Table).

Animals in the CA group developed pronounced hypercapnia, hyperlactatemia, and an increase in base deficit as well as decompensated mixed acidosis by the 10th minute of the post-resuscitation period. Furthermore, hyperoxemia was detected in animals from the CA group compared to the SO group with the mechanical ventilation with 100% oxygen fraction, while the oxygenation index (p/F) was reduced (Table). Two hours after return of spontaneous circulation, there was no statistical difference in ABB or blood gases between the groups (except for higher blood oxygenation in the CA group), demonstrating the efficacy of intensive therapy and mechanical ventilation in the CA group (Table). All surviving animals in the CA group showed spontaneous respiration 2 h after blood circulation was restored after cessation of mechanical ventilation, with underlying depressed consciousness.

Experimental models of asphyxial CA in laboratory animals better reproduce the mechanisms

Table. Circulation parameters. blood gas and acid-base balance of rats at baseline and in the early post-resuscitation period; *Me (LQ; UQ)*.

Parameter	Baseline			5–10 min of the post-resuscitation period			115–120 min of the post-resuscitation period		
	SO group, *N*=11	CA group, *N*=18	*P*-value	SO group, *N*=11	CA group, *N*=18	*P*-value	SO group, *N*=11	CA group, *N*=15	*P*-value
BP_{mean}, mmHg	70 [65; 78]	72 [65; 77]	0.912	60 [58; 72]	67 [62; 82]	0.482	63 [57; 68]	63# [62; 67]	0.892
HR, per minute	222 [210; 231]	238 [217; 253]	0.159	218 [205; 236]	286 [272; 305]	0.0001	232 [206; 257]	247 [236; 261]	0.055
M, pfu	14.8 [13.0; 16.5]	15.9 [13.4; 17.4]	0.610	14.7 [12.1; 16.5]	10.1# [7.0; 12.5]	0.0014	15.4 [13.0; 17.4]	14.9 [13.2; 16.1]	0.443
pH	7.41 [7.38; 7.43]	7.43 [7.40; 7.50]	0.674	7.42 [7.40; 7.43]	7.20# [7.13; 7.23]	<0.0001	7.44 [7.40; 7.46]	7.47 [7.33; 7.53]	0.437
$PaCO_2$, mmHg	37.8 [34.1; 41.3]	38.4 [34.7; 41.9]	0.991	37.8 [32.7; 42.4]	51.3# [41.2; 60.9]	0.0001	34.7 [31.5; 39.7]	33.9# [30.3; 52.9]	0.861
PaO_2, mmHg	70 [59; 86]	69 [63; 79]	0.851	78 [64; 91]	120# [83; 139]	0.0045	82 [75; 91]	291# [194; 376]	0.0001
BE, mmol/L	0 [–1; 1]	1 [–1.5; 3]	0.554	–0.5 [–2.7; 1.7]	–9# [–11; –8]	<0.0001	–1.0 [–2.0; 0.5]	–2.0 [–4.0; 3.0]	0.965
Lactate, mmol/L	1.16 [1.02; 1.53]	1.16 [0.88; 1.65]	0.782	1.13 [1.52; 5.42]	5.58# [4.65; 6.90]	<0.0001	1.21 [0.95; 1.94]	1.47 [1.16; 2.57]	0.473
SaO_2. %	93 [90; 97]	94 [92; 96]	0.974	95 [93; 97]	98 [93; 99]	0.087	96 [95; 97]	100# [99; 100]	0.0001
HCO_3, mmol/L	24.2 [23.4; 25.4]	25.4 [23.1; 26.9]	0.588	24.2 [17.1; 25.6]	18.4# [17.2; 20.8]	0.0002	23.3 [20.3; 24.6]	24.3# [21.0; 25.8]	0.650
p/F	333 [297; 404]	337 [314; 385]	0.991	337 [285; 390]	121# [93; 136]	<0.0001	390 [358; 425]	295# [202; 359]	0.0338

Note. BP_{mean} — mean blood pressure; HR — heart rate; M — mean perfusion value; $PaCO_2$ — partial pressure of CO_2 in the arterial blood; PaO_2 — partial pressure of O_2 in the arterial blood; BE — base excess; SaO_2 — oxygen saturation of arterial blood; HCO_2 — level of bicarbonate; p/F — oxygenation index. *P* — exact *P*-value for SO vs CA; # — $P<0.05$ vs baseline (with Bonferroni correction for multiple comparisons).

of cardiac arrest due to extracardiac causes in patients with different profiles and are increasingly used in modern experimental medicine [12].

The literature search revealed a number of methodological solutions for modeling asphyxial cardiac arrest, as well as the identification and evaluation of their advantages and disadvantages. Anesthetic agents include both inhalational (sevoflurane and isoflurane) [20, 21] and non-inhalational (pentobarbital and chloral hydrate) [15, 22]. When inhalational anesthetics are used, anesthesia is discontinued before asphyxia is induced. Methodologically, this reduces the effect of anesthesia on the pathogenesis of ischemia-reperfusion injury as a component of multiorgan dysfunction [23], but it does not reflect the pathogenesis of CA in clinical anesthesiology.

In this study, we used a combination of the NMDA receptor antagonist tiletamine/zolazepam and the central α2-adrenoreceptor agonist xylazine (veterinary analog of dexmedetomidine) to achieve minimal cardiorespiratory effects, sufficient depth of anesthesia, and myorelaxation for invasive manipulations [16].

Despite the similarities in the methods used by different authors to model asphyxial CA, a review of literature sources reveals a significant heterogeneity of models, implying insufficient comparability and reproducibility of the obtained experimental data [24].

In general, an evaluation of the methodological approaches used in existing models allows us to identify a number of common shortcomings. Most experimental models use specific BP values to calculate the moment of CA, which vary widely between sources (from 10 mm Hg to 30 mm Hg) [15, 25]. At the same time, the authors do not assess organ and tissue perfusion, and the duration of CA is often determined by the total duration of asphyxia rather than CA itself, which reduces standardization of the severity of ischemia-reperfusion injury.

To confirm the time of cardiac arrest and return of spontaneous circulation after resuscitation, we used the LDF method to assess skin perfusion in conjunction with ECG and blood pressure monitoring. The duration of cardiac arrest was measured from the moment of EMD using the following criteria: BP_{mean} drop below 20 mmHg for at least 10 seconds, marked bradycardia (less than 100/min in rats) or other agonal heart rhythm (idioventricular rhythm, blocks, etc.), and skin perfusion at the «biological zero» LDF level.

In various studies, supportive therapy in the post-resuscitation period was limited in time and volume and did not correspond to modern resuscitation protocols (insufficient duration of ventilation, lack of clear protocols for the use of vasopressors and inotropes, lack of protocols for volemic support and ABB correction, etc.). These features limit the translational potential of preclinical study results for clinical anesthesiology and resuscitation [26–27]. When we performed supportive intensive therapy in the post-resuscitation period of the study, the

BPmean values did not show statistical differences between the groups at either 10 or 120 minutes, and the mixed acidosis observed in the CA group was compensated by 120 minutes, indicating the effectiveness of intensive therapy.

Thus, the results of the experiments showed that the use of prolonged non-inhalational combined anesthesia, documentation of CA onset and restoration of spontaneous circulation using LDF, performing comprehensive intensive care in the post-resuscitation period for at least 2 hours, improved the quality of modeling of asphyxial CA by reducing the variability of functional parameters of animals, improving the reproducibility of the model and its similarity to the post-resuscitation disease in humans in the clinical setting. This improvement was achieved using methodological techniques described in the literature. In particular, we considered the experience of modeling asphyxial CA with resuscitation to evaluate the neuroprotective effects of hypothermia and glibenclamide as described by Huang et al. [17].

A limitation of this study is the use of non-inhalational anesthetics to induce anesthesia. Despite several advantages over inhalational anesthetics (low severity of cardiorespiratory manifestations, sufficient depth of sedation and myorelaxation for surgical manipulations, and a sufficient degree of analgesia in the experimental animal), the use of combined anesthesia reduces the similarity of the model to out-of-hospital CA and may also contribute to the bias of experimental data from the perspective of drug property studies. It is also necessary to consider the toxic effects of oxygen and to limit the duration of high fractional oxygen ventilation [28]. Furthermore, the modeling of asphyxial CA in the current variant is a technically demanding process that requires both the use of specialized equipment and the development of appropriate investigator skills. The study of remote (3–7 days and longer) outcomes of post-resuscitation disease in experimental animals with longer duration of CA (4–8 min) is of great clinical importance, but raises additional organizational (need for prolonged intensive care) and bioethical concerns.

Conclusion

Cardiac arrest in rats during asphyxia under general anesthesia occurs via electromechanical dissociation. Separate documentation of the time of respiratory and cardiac arrest and using LDF to assess peripheral blood flow allows standardization of the severity of ischemia-reperfusion injury and improves model reproducibility. The use of a set of resuscitation measures that fit modern standards of patient management in clinical anesthesiology and intensive care is bioethically sound and allows for better translation of preclinical study results into clinical medicine.

References

1. *Nolan J. P., Sandroni C., Böttiger B. W., Cariou A., Cronberg T., Friberg H., Genbrugge C., et al.* European Resuscitation Council and European Society of Intensive Care Medicine guidelines 2021: post-resuscitation care. *Intensive Care Med.* 2021; 47 (4): 369–421. DOI: 10.1007/s00134-021-06368-4. PMID: 33765189.
2. *DiLibero J., Misto K.* Outcomes of in-hospital cardiac arrest. *Crit Care Nurs Cli North Am.* 2021; 33 (3): 343–356. DOI: 10.1016/j.cnc.2021.05.009. PMID: 34340795
3. *Andersen LW, Holmberg M. J., Berg K. M., Donnino M. W., Granfeldt A.* In-hospital cardiac arrest: a review. *JAMA.* 2019; 321 (12): 1200–1210. DOI: 10.1001/jama.2019.1696. PMID: 30912843.
4. *Николовски С. С., Божич Н. Б., Фишер З., Лазич А. Д., Тиянич Е. З., Раффэй В. И.* Влияние сердечно-легочной реанимации с поддержкой диспетчером скорой медицинской помощи на восстановление эффективного кровообращения и краткосрочную выживаемость. *Общая реаниматология.* 2021; 17 (5): 52–64. *Nikolovski S. S., Bozic N. B., Fiser Z. Z., Lazic A. D., Tijanic J. Z., Raffay V. I.* Dispatcher-assisted cardiopulmonary resuscitation — influence on return of spontaneous circulation and short-term survival. *General Reanimatology=Obshchaya Reanimatologya.* 2021; 17 (5): 52–64. (in Russ.&Eng.). DOI: 10.15360/1813-9779-2021-5-52-64.
5. *Virani S. S., Alonso A., Benjamin E. J., Bittencourt M. S., Callaway C. W., Carson A. P., Chamberlain A. M., et al.* Heart disease and stroke statistics-2020 update: a report from the American Heart Association. *Circulation.* 2020; 141 (9): e139–e596. DOI: 10.1161/CIR.0000000000000757. PMID: 31992061
6. *Patel K. K., Spertus J. A., Khariton Y., Tang Y., Curtis L. H., Chan P. S., AHA Get with the Guidelines — resuscitation investigators.* Association between prompt defibrillation and epinephrine treatment with long-term survival after in-hospital cardiac arrest. *Circulation.* 2018; 137 (19): 2041–2051. DOI: 10.1161/CIRCULATIONAHA.117.030488. PMID: 29279412.
7. *Мороз В. В., Бобринская И. Г., Васильев В. Ю., Кузовлев А. Н., Перепелица С. А., Смелая Т. В., Спиридонова Е. А., с соавт.* Сердечно-легочная реанимация. Москва: ФНКЦ РР, МГМСУ, НИИОР. 2017. *Moroz V. V., Bobrinskaya I. G., Vasiliev V.Yu., Kuzovlev A. N., Perepelitsa S. A., Smelaya T. V., Spiridonova E. A., et al.* Cardiopulmonary resuscitation. Moscow: FSCC ICR, MSMSU, RI GR, 2017. (in Russ.). ISBN 978-5-9500558-0-5.
8. *Неговский В. А.* Очерки по реаниматологии. Москва: Медицина; 1986. *Negovsky V. A.* Essays on intensive care. Moscow: Meditsina; 1986. (in Russ.).
9. *Rajan S., Folke F., Hansen S. M., Hansen C. M., Kragholm K., Gerds T. A., Lippert F. K., et al.* Incidence and survival outcome according to heart rhythm during resuscitation attempt in out-of-hospital cardiac arrest patients with presumed cardiac etiology. *Resuscitation.* 2017; 114: 157–163. DOI: 10.1016/j.resuscitation.2016.12.021. PMID: 28087286.
10. *Varvarousis D., Varvarousi G., Iacovidou N., D'Aloja E., Gulati A., Xanthos T.* The pathophysiologies of asphyxial vs dysrhythmic cardiac arrest: implications for resuscitation and post-event management. *Am J Emerg Med.* 2015; 33 (9): 1297–304. DOI: 10.1016/j.ajem.2015.06.066. PMID: 26233618
11. *Брагина Н. В., Маркова Т. Г., Горбачев В. И.* Постреанимационная болезнь. *Анестезиология и реаниматология.* 2021; (4): 140–150. *Bragina N. V., Markova T. G., Gorbachev V. I.* Post-resuscitation disease. *Anesthesiol.Reanimatol=Anesteziologiya i Reanimatologiya.* 2021; (4): 140–150. (in Russ.). DOI: 10.17116/anaesthesiology2021041140.
12. *Yu S., Wu C., Zhu Y., Diao M., Hu W.* Rat model of asphyxia-induced cardiac arrest and resuscitation. *Front Neurosci.* 2023; 16: 1087725. DOI: 10.3389/fnins.2022.1087725. PMID: 36685224.
13. *Katz L., Ebmeyer U., Safar P., Radovsky A., Neumar R.* Outcome model of asphyxial aardiac arrest in rats. *J Cereb Blood Flow Metab.* 1995; 15 (6): 1032–1039. DOI: 10.1038/jcbfm.1995.129. PMID: 7593335
14. *Dave K. R., Raval A. P., Prado R., Katz LM., Sick T. J., Ginsberg M.D., Busto R., et al.* Mild cardiopulmonary arrest promotes synaptic dysfunction in rat hippocampus. *Brain Res.* 2004; 1024 (1–2): 89–96. DOI: 10.1016/j.brainres.2004.07.050. PMID: 15451369.
15. *Hu T., Wang J., Wang S., Li J., Chen B., Zuo F, Zhang L., et al.* Effects of the duration of postresuscitation hyperoxic ventilation on neurological outcome and survival in an asphyxial cardiac

arrest rat model. *Sci Rep*. 2019; 9 (1): 16500. DOI: 10.1038/s41598-019-52477-y. PMID: 31712629.

16. *Дубенский А. Ю., Рыжков И. А., Лапин К. Н., Цоколаева З. И., Калабушев С. Н., Варнакова Л. А., Долгих В. Т., с соавт.* Влияние вида анестезии на показатели кровообращения у крыс. *Вестник СурГУ. Медицина*. 2023; 16 (2): 79–86. *Dubensky A.Yu., Ryzhkov I. A., Lapin K. N., Tsokolaeva Z. I., Kalabushev S. N., Varnakova L. A., Dolgikh V. T., et al.* Influence of anesthesia type on the blood circulation in rats. *Bulletin of SurGU. Medicine=Vestnik SurGU. Meditsina*. 2023; 16 (2): 79–86 (in Russ.). DOI: 10.35266/2304-9448-2023-2-79-86.
17. *Huang K., Wang Z., Gu Y., Hu Y., Ji Z., Wang S., Lin Z.,et al.* Glibenclamide Is comparable to target temperature management in improving survival and neurological outcome after asphyxial cardiac arrest in rats. *J Am Heart Assoc*. 2016; 5 (7): e003465. DOI: 10.1161/JAHA.116.003465. PMID: 27413041.
18. *Лапин К. Н., Рыжков И. А., Мальцева В. А., Удут Е. В.* Катетеризация сосудов мелких лабораторных животных при проведении биомедицинских исследований: технологические аспекты метода: обзор. *Бюллетень сибирской медицины*. 2021; 20 (3): 168–181. *Lapin K. N., Ryzhkov I. A., Maltseva V. A., Udut E. V.* Vascular catheterization of small laboratory animals during biomedical research: technological aspects of the method: review. *Bulletin of Siberian Medicine=Bulleten Sibirskoy Meditsiny*. 2021; 20 (3): 168–181 (in Russ.). DOI 10.20538/1682-0363-2021-3-168-181.
19. *Wang-Fischer Y.* (ed.). Manual of stroke models in rat. Boca Raton: CRC Press; 2009: 332.
20. *Keilhoff G., Esser T., Titze M., Ebmeyer U., Schild L.* High-potential defense mechanisms of neocortex in a rat model of transient asphyxia induced cardiac arrest. *Brain Res*. 2017; 1674: 42–54. DOI: 10.1016/j.brainres.2017.08.018. PMID: 28827077.
21. *Tungalag T., Yoo Y.-J., Tae H.-J., Yang D. K.* Olanzapine-induced therapeutic hypothermia attenuates renal injury in rats after asphyxial cardiac arrest and resuscitation. *Antioxidants (Basel)*. 2022; 11 (3): 443. DOI: 10.3390/antiox11030443. PMID: 35326094.
22. *Zhou X., Liu,Y., Huang Y., Zhu S., Zhu J., Wang R.* Hypertonic saline infusion suppresses apoptosis of hippocampal cells in a rat model of cardiopulmonary resuscitation. *Sci. Rep*. 2017; 7 (1): 5783. DOI: 10.1038/s41598-017-05 919-4. PMID: 28724904.
23. *Murakami M., Niwa H., Kushikata T., Watanabe H., Hirota K, Ono K., Ohba T.* Inhalation anesthesia is preferable for recording rat cardiac function using an electrocardiogram. *Bio Pharm Bull*. 2014; 37 (5): 834–839. DOI: 10.1248/bpb.b14-00012. PMID: 24790005.
24. *Vognsen M., Fabian-Jessing B. K., Secher N., Løfgren B., Dezfulian C., Andersen L. W., Granfeldt A.* Contemporary animal models of cardiac arrest: a systematic review. *Resuscitation*. 2017; 113: 115–123. DOI: 10.1016/j.resuscitation.2017.01.024. PMID: 28214538.
25. *Uray T., Dezfulian C., Palmer A. A., Miner K. M., Leak R. K., Stezoski J. P., Janesko-Feldman K.* Cardiac arrest induced by asphyxia versus ventricular fibrillation elicits comparable early changes in cytokine levels in the rat brain, heart, and serum. *J Am Heart Assoc*. 2021; 10 (5): e018657. DOI: 10.1161/JAHA.120.018657. PMID: 33599149.
26. *Nolan J. P., Soar J., Cariou A., Cronberg T., Moulaert V. R.M., Deakin C. D., Bottiger B. W.* European resuscitation Council and European Society of Intensive Care Medicine guidelines for post-resuscitation care 2015: Section 5 of the European resuscitation Council guidelines for post-resuscitation care 2015. *Resuscitation*. 2015; 95: 202–222. DOI: 10.1016/j.resuscitation.2015.07.018. PMID: 26477702.
27. *Diao M.-Y., Zheng J., Shan Y., Xi S., Zhu Y., Hu W., Lin Z.* Hypothermia prevents hippocampal oxidative stress and apoptosis via the GSK-3beta/Nrf2/HO-1 signaling pathway in a rat model of cardiac arrest-induced brain damage. *Neurol. Res*. 2020; 42 (9): 773–782. DOI: 10.1080/ 01616412. 2020.177 4210. PMID: 32529954.
28. *Долгих В. Т., Говорова Н. В., Орлов Ю. П., Корпачева О. В., Доровских Г. Н., Ершов А. В.* Патофизиологические аспекты гипероксии в практике анестезиолога реаниматолога

(мини-обзор). *Общая реаниматология*. 2017; 13 (3): 83–93. *Dolgikh V. T., Govorova N. S. Orlov Yu.P., Korpacheva O. S., Dorovskikh G. N. Yershov A. S.* Pathophysiological aspects of hyperoxia in anesthesiologist-reanimatologist's practice. *General Reanimatology=Obshchaya Reanimatologya*. 2017; 13 (3): 83–93. (in Russ.&Eng.). DOI: 10.15360/1813-9779-2017-3-83-93.

Received 13.11.2023
Accepted 20.02.2024