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Inhalation of argon-krypton gas mixture improves survival in cases of acute massive blood loss: a randomized study in pigs

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Abstract

INTRODUCTION: Acute massive blood loss is a critical condition associated with the loss of a significant volume of circulating blood, leading to hemorrhagic shock and multiorgan failure. In the setting of prehospital stage and scarce resources carrying out early and adequate intensive care may be impossible or significantly delayed. In these cases reduced oxygen delivery to tissues leads to severe ischemia, and cellular resistance to hypoxic damage becomes one of the key factors determining poor outcomes. Thus, in recent years special attention was given to cytoprotective properties of noble gases. **OBJECTIVE:** To study the effect of using respiratory mixtures with elevated concentrations of noble gases (argon and krypton) on survival in acute massive blood loss. **MATERIALS AND METHODS:** A prospective randomized controlled study was conducted on 16 pigs (35–50 kg). Experimental animals were randomized into 2 groups: a control group ($n = 8$) (30 % oxygen-air mixture) and an experimental group ($n = 8$) ("Argon-Krypton" mixture: 60 % argon, 30 % oxygen, and 10 % krypton). Acute massive blood loss up to 50 % of the total blood volume was simulated without infusion therapy. Animal survival over 2 hours was assessed, and hemodynamics, acid-base balance, arterial blood gas composition, and hematological parameters were monitored. **RESULTS:** In the experimental group, 1 animal died, compared to 3 animals in the control group ($p < 0.001$). Following blood loss simulation the experimental group showed higher rates of mean arterial pressure 57 (53; 66) *vs*. 39 (20; 51) mm Hg, in the control group ($p = 0.041$), partial pressure of oxygen 132 (90; 146) *vs*. 84 (76; 94) mm Hg ($p = 0.028$) and arterial blood oxygen saturation 99 (97; 99) *vs*. 96 (94; 97) % ($p = 0.015$).

ФУНДАМЕНТАЛЬНЫЕ ВОПРОСЫ ИНТЕНСИВНОЙ ТЕРАПИИ

Ингаляция газовой смеси с аргоном и криптоном увеличивает выживаемость при острой массивной кровопотере: рандомизированное исследование на свиньях

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Реферат

АКТУАЛЬНОСТЬ: Острая массивная кровопотеря (ОМК) представляет собой критическое состояние, связанное с потерей значительного объема циркулирующей крови (ОЦК), приводящее к геморрагическому шоку и полиорганной недостаточности. В условиях догоспитального этапа и ограниченных ресурсов проведение своевременной и полноценной интенсивной терапии может быть невозможно или существенно отсрочено. В подобных ситуациях уменьшение доставки кислорода к тканям приводит к выраженной ишемии, а устойчивость клеток к гипоксическому повреждению становится одним из ключевых факторов неблагоприятных исходов. В связи с этим в последние годы особое внимание уделяется цитопротективным свойствам инертных газов. **ЦЕЛЬ ИССЛЕДОВАНИЯ:** Изучить влияние применения дыхательной смеси с повышенным содержанием инертных газов (аргона и криптона) на выживаемость при ОМК. **МАТЕРИАЛЫ И МЕТОДЫ:** Проспективное рандомизированное контролируемое исследование на 16 свиньях (35–50 кг). Экспериментальные животные были рандомизированы на 2 группы: контрольная группа ($n = 8$) (30 % кислородно-воздушная смесь) и опытная группа ($n = 8$) (дыхательная смесь «Аргон-криптон»: 60 % аргона; 30 % кислорода и 10 % криптона). Моделировали ОМК до 50 % ОЦК без проведения инфузионной терапии. Оценивали выживаемость животных в течение 2 ч, мониторировали гемодинамику, изменения кислотно-основного равновесия, газового состава артериальной крови и гематологические показатели. **РЕЗУЛЬТАТЫ:** В опытной группе погибло 1 животное, в контрольной группе — 3 животных ($p < 0,001$). После

Acid-base status remained stable in the experimental group, whereas progressive metabolic acidosis was observed in the control group; statistically significant intergroup differences were found within 2 hours of monitoring (p = 0.005). **CONCLUSIONS:** The use of a gas mixture containing argon and krypton in acute massive blood loss is associated with increased survival in large laboratory animals (pigs), promotes systemic hemodynamics stabilization and reduces the severity of metabolic acidosis.

моделирования кровопотери в опытной группе регистрировали более высокие значения среднего артериального давления (57 [53; 66] *vs* 39 [20; 51] мм рт. ст. в контрольной; p = 0,041), парциального давления кислорода (132 [90; 146] *vs* 84 [76; 94] мм рт. ст.; p = 0,028) и сатурации артериальной крови (99 [97; 99] *vs* 96 [94; 97] %; p = 0,015). Кислотно-основное состояние в опытной группе оставалось стабильным, тогда как в контрольной группе наблюдалось прогрессирование метаболического ацидоза; через 2 ч наблюдения выявлены статистически значимые межгрупповые различия (p = 0,005). **ВЫВОДЫ:** Применение газовой смеси с аргоном и криптоном при ОМК сопровождается повышением выживаемости крупных лабораторных животных (свиней), способствует стабилизации системной гемодинамики и снижению выраженности метаболического ацидоза.

KEYWORDS: hemorrhagic shock, survival rate, argon, krypton, oxygen, animals

КЛЮЧЕВЫЕ СЛОВА: геморрагический шок, выживаемость, аргон, криптон, кислород, животные

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Introduction

Acute massive blood loss (AMBL) is a life-threatening condition, characterized by 40 % and more loss of circulating blood volume (BV) that commonly results in complications such as hemorrhagic shock, multiorgan failure with high likelihood of lethal outcome. Every year more than 5.8 million people die of severe injuries and their consequences, AMBL prevailing among them [1–5].

In situations associated with AMBL, adequate oxygen delivery to cells is critically important. The study shows that monitoring of mixture proportion of respiratory gas during acute period of traumatic disease can have a significant effect on metabolic processes occurring in the body, and hence, expected outcome for patients suffering from severe trauma and blood loss [6–8]. In case of blood loss leading to BV reduction and organs and tissues hypoxia, a cascade of pathophysiological processes develops, which include activation of inflammatory reactions and oxidative stress. Oxidative stress caused by free radical excess can result in cell structure damage which includes lipids, proteins and nucleic acids, which in turn can promote cell apoptosis and necrosis [9, 10]. Cytoprotective properties of noble gases, such as argon and krypton, when used in the case of blood loss, present a new area of exploration in pathophysiology and clinical medicine. These gases possessing unique physicochemical nature demonstrate a number of biological effects facilitating reduced cell damage and improved functional outcome in the presence of acute massive blood loss and its complication in the form of hemorrhagic shock [11].

Argon has an antioxidant effect, reducing free radicals formation and protecting cells from oxidative stress [12]. The studies showed that this noble gas can have a protective action on neurons during hypoxia [13, 14]. In cases when brain blood supply is temporarily disturbed, the use of argon can promote reducing cell damage and prevention of neuronal death [15]. The mechanisms of action of krypton are not fully understood, however, the studies demonstrated its cytoprotective, anxiolytic and analgesic activity [16–18].

Thus, the use of air mixtures with high noble gases concentration can be beneficial in reducing cell damage and improving patients' outcomes.

Objective

To study the effect of respiratory mixtures with high concentration of noble gases (argon and krypton) on survival rates in cases of acute massive blood loss.

Materials and methods

Contents and ethical considerations

This study was approved by the local independent Ethical Committee at the Kirov Military Medical Academy (reference number 279-27.06.2023).

The experimental work was conducted subject to the rules of animal care and use in accordance with the Russian Federation law "On Veterinary Medicine" № 4979-1 of 14.05.1993, GOST 33215-2014, and recommendations presented by Board of the Eurasian Economic Commission № 33 of 14.11.2023 "Guidelines for Dealing with Laboratory (Experimental) Animals in the Process of Nonclinical Studies".

Before the study began, the animals were kept in a box for one day for the adaptation. At first, visual examination of the animals was carried out in order to exclude sick and weak animals. The experiment exclusion was performed in the setting of continuing anesthesia.

The study design

Controlled prospective randomized study on experimental animals was carried out at the base of the Kirov Military Medical Academy, Ministry of Defense, the Russian Federation, in the period from September to November 2024.

The study of noble gas mixture "argon-krypton" was carried out on 16 big animals — pigs of one breed, weighing 35–50 kg. The size of the sample was calculated with the help of specialized software (G*Power 3.1, Germany). The calculation was based on large animal survival rate during 120 minutes. Considering the findings of previous studies the expected effect (d) amounted to 1.45. The power capacity being 80 % and significance level being $\alpha = 0.05$, the necessary sample size was 8 animals in a group (total 16 animals). For AMBL simulation the animals were randomized into two groups, 8 animals in each. Randomization sequence was formed using random number generator. Oxygen-air mixture with O_2 concentration —30 % was used in the control group. Respiratory mixture containing high noble gases concentration (argon = 60 %, oxygen = 30 %, krypton = 10 %) was used in the experimental group.

Medical treatment description

In the setting of an operating room anaesthesia was induced by intramuscular administration of the drug "Zoletil R 100" (dose of 20 mg/kg of animal weight) injected into the thigh. The trachea was intubated at the operating room while the animal was in a prone position in order to prevent aspiration and regurgitation. Then the animal was placed into a dorsal position, and ultrasound guided femoral cannulation according to Seldinger method using the introducers 6Fr was carried out. Catheters installed were used for invasive blood pressure monitoring, blood loss simulation, as well as for blood sampling in laboratory studies. Then gas mixture supply was delivered using the mechanical respirator Mindray WATO EX-35 (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China) in the appropriate group. Gas mixture "argon-krypton" was supplied to the artificial respirator breathing circuit using the device "Lifeguard-mix" (respiratory gas mixer, LLC "Research Institute GEROPRO", Russia), connected to gas balloon filled with "argon-krypton" mixture (argon 60 %, oxygen 30 %, krypton 10 %) and to balloon filled with medical oxygen. The mixer was connected to the artificial respirator breathing circuit providing dosed gas supply to the circuit with the rate of supply up to 15 L/min while maintaining preset parameters of component concentration in the inhaled mixture. Gas mixture composition was controlled by means of gas detector built into the device "Life-guard — mix", calibrated to be used with a given mixture.

Blood loss simulation was carried out with the help of a plasmapheresis device "Gemma" (Russia). Not less than 45 % and not more than 50 % of BV was collected through left femoral artery, taking into consideration systolic arterial pressure decrease in the animal down the rates not lower than 50 mm Hg. Total blood loss volume in ml and % versus total BV was calculated according to the formula:

$$V_{BV} = M \times 0.07,$$

where: V_{BV} — circulating blood volume; ml; M — animal weight, gr.

The rates of systolic, diastolic and mean arterial blood pressure (MAP), heart rate (HR), respiratory rate were recorded at 6 points before blood loss, during blood loss of 20 % of BV, 45 % of BV in 30, 60 and 120 minutes after blood loss. At the same time, venous blood sampling for clinical assessment of blood, blood gases, acid-base balance and arterial lactate concentration was carried out. Gas mixture inhalation was continued for 2 hours after blood loss, followed by euthanasia and postmortem examination of animals.

Outcome registration procedure

Survival assessment of animals was considered to be a primary endpoint. Secondary endpoints were clinical assessment of hemodynamics indices (MAP, HR), the analysis of difference in hematologic markers dynamics (total erythrocytes, thrombocytes, HGB level, hematocrit), blood-gas tension ($PaCO_2$ and PaO_2), arterial saturation (SaO_2), acid-base balance (blood pH), base deficit (BE), arterial bicarbonate (HCO_3^-) and lactate concentration.

Recording of systemic hemodynamics (arterial blood pressure, HR) was performed with the help of the sensor system to monitor vital animals functions. Gas composition and arterial acid-base balance as well as blood lactate was measured with operative analyzer "VetScan i-STAT 1" (Abbott, USA). General blood test was made with veterinary hematology analyzer MicroCC-20 Plus (veterinary version) (High Technology, Inc., USA).

Statistical analysis

Data collection and statistical analysis were made with Microsoft Excel software (Microsoft Corp., USA) and Statistica 7 and 10 (StatSoft Inc, USA). The median value (Me) and lower quartile Q1 (25 %) and upper quartile Q3 (75 %) values presented here as Me (Q1; Q3), were used as descriptive statistics due to limited sample size. Assessment of statistical significance was performed in accordance with Mann-Whitney, Moses, Friedman test and Wilcoxon signed-rank test. Survival of the groups is presented using Kaplan-Meier curve. The comparison of survival curves was carried out with the help of log-rank test and Peto & Peto's modified Wilcoxon test. The level of statistically significant difference was determined as $\alpha = 0.05$. In multiple intra-group comparison procedures of points being studied (background, 50 % BV, 120 minutes after AMBL simulation) Wilcoxon T-criterion was applied with Bonferroni adjustment ($\alpha = 0.025$).

Results

Primary endpoint

Survival assessments showed that the best results were recorded in the experimental group. One animal died in the experimental group during the experiment, while 3 animals died in the control group. Survival function based on Kaplan-Meier curves is presented on the diagram (Figure 1). According to log-rank test and Peto & Peto's modified test there was no statistical difference between these groups found. However, taking into account comparison of critical outcomes in the control and experimental groups, Moses criterion was used that showed the presence of statistical differences ($p < 0.001$). Relative risk of the development of unfavorable outcome in the control group was 3 times higher (RR 95 %) = 3.0 (0.390–23.073) with sensitivity 0.750 and specificity 0.583.

Secondary endpoint

In the experiment, arterial pressure decrease was found in the setting of persistent blood loss. MAP dynamics is shown on the diagram (Figure 2). In AMBL simula-

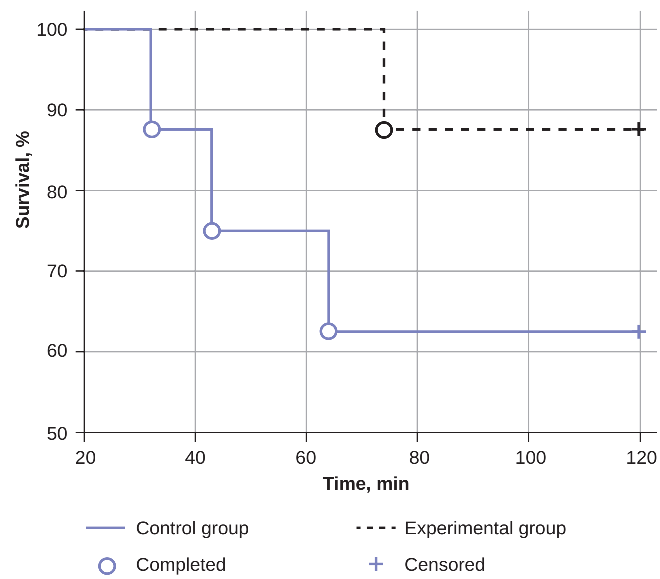

Fig. 1. Animal survival within 120 minutes after acute massive blood loss simulation

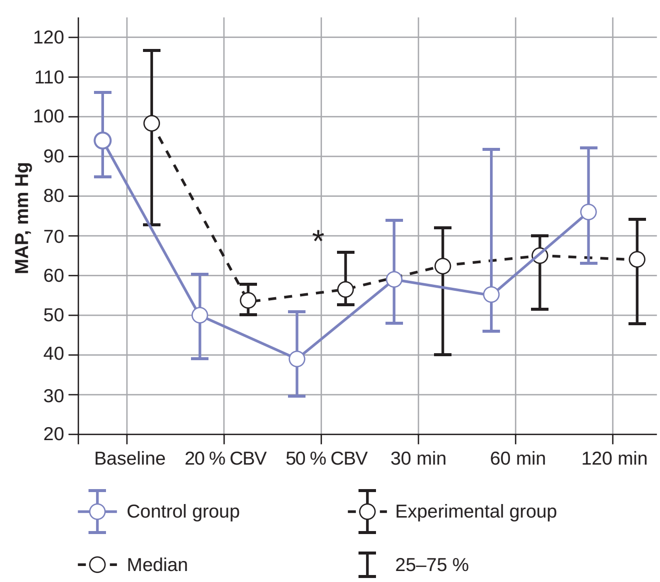

Fig. 2. Mean arterial pressure dynamics in the experiment

Note: CBV — circulating blood volume; MAP — mean arterial pressure.

* Statistically significant differences.

tion process there was no blood loss compensation done, hence there was significant decrease of MAP in both groups (from initial level to blood loss of 50 % BV): in the experimental group it decreased from 99 (74; 117) down to 57 (53; 66) mm Hg ($p = 0.012$), in the control group — from 94 (85; 106) down to 39 (20; 51) mm Hg ($p = 0.018$). Yet, statistical analysis demonstrated, that MAP at the end of blood loss process was much higher in the group where noble gases were inhaled in contrast to the control group ($p = 0.041$). At the stages of 30, 60 and 120 minutes after blood loss significant differences between groups were not registered.

When arterial pressure decreased in both groups, compensatory increase of HR was observed (Figure 3). At the same time, in the control group animals demonstrated more pronounced tachycardia, especially 1 hour after AMBL, when differences became statistically significant ($p = 0.008$). In the experimental group HR increased by the end of monitoring period from 86 (74; 107) up to 165 (161; 198) beats per minute, while in the control group — from 102 (84; 145) up to 226 (210; 233) beats per minute.

Comparative analysis of laboratory findings in both study groups at different stages of experiment is shown in Table 1.

Arterial pH dynamics in both groups is shown in the diagram (Fig. 4). Primary rates were within the reference limits (7.330–7.480) and statistically did not differ in both groups ($p > 0.05$). In 60 minutes after AMBL simulation significant pH decrease was recorded from 7.386 (7.350; 7.414) down to 7.301 (7.281; 7.341); $p = 0.042$ in the control group. Significant pH decrease from 7.460 (7.330; 7.495) down to 7.348 (7.330; 7.350); $p = 0.043$ was also registered in the experimental group.

The endpoint monitoring in the control group (oxygen-air mixture) showed that the level of pH remained at the level of pronounced acidosis — 7.299 (7.277; 7.356), whereas in the experimental group there was tendency of incremental recovery — 7.380 (7.377; 7.430). The differences between groups were statistically significant ($p = 0.005$). In both animal groups increased arterial lactate concentration occurred (Table 1). More pronounced increase in lactatemia level was recorded in case of 50 % BV loss in the control group from 1.91 (1.49; 2.91) to 6.10 (5.43; 7.71) mmol/l ($p = 0.012$). At this stage intergroup differences were statistically significant ($p = 0.005$).

After 120 minutes of monitoring lactate concentration maintained the stable high level in the control group. At the same time gradual increase in the lactate level up to the values comparable with control ones, — 7.65 (3.42; 11.69) mmol/l were recorded (at this stage statistically significant differences between groups were not found — $p > 0.05$).

Primary rates of O_2 partial pressure were comparable within the groups (Table 1). However, when BV was decreasing to 50 % statistically significant intergroup differences were detected ($p = 0.028$). In the experimental group PaO_2 remained stable during the period of monitoring ($p > 0.05$), while in the control group it decreased considerably from 110 (94; 150) mm Hg down to 84 (79; 93) mm Hg ($p = 0.027$). Intergroup differences of SaO_2 also were statistically significant ($p = 0.015$) and were compliant with PaO_2 dynamics (Table 1).

To compare the groups under study the following data in the complete blood count were used: total erythrocyte count, hemoglobin level, hematocrit, total thrombocyte count, their dynamics being presented in the table

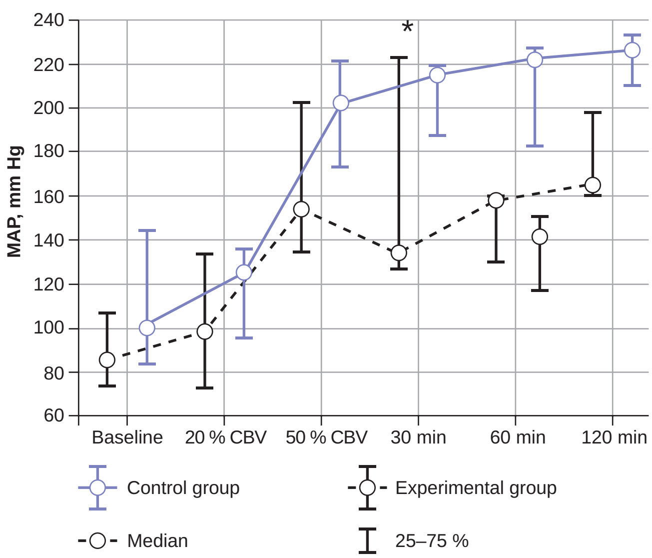

Fig. 3. Heart rate dynamics in the experiment

Note: CBV — circulating blood volume.

* Statistically significant differences.

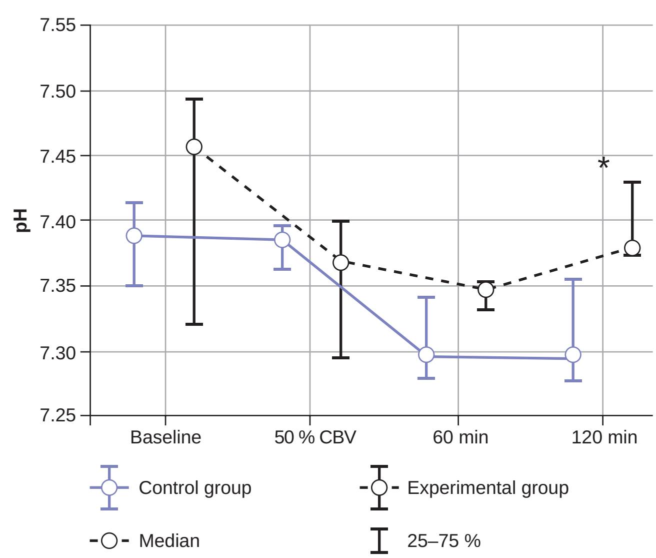

Fig. 4. Changes in arterial blood pH during the experiment

Note: CBV — circulating blood volume.

* Statistically significant differences.

Table 1. Comparative analysis of laboratory findings at different stages of the experiment

Parameter	Baseline			50 % BV			60 min			120 min		
	Experimental group	Control group	*p*-value	Experimental group	Control group	*p*-value	Experimental group	Control group	*p*-value	Experimental group	Control group	*p*-value
pH	7.460 (7.330; 7.495)	7.386 (7.350; 7.414)	0.442	7.370 (7.301; 7.400)	7.383 (7.363; 7.395)	0.645	7.348 (7.330; 7.350)	7.301 (7.281; 7.341)	0.414	7.380 (7.377; 7.430)	7.299 (7.277; 7.356)	**0.005**
$PaCO_2$, mm Hg	45.0 (38.0; 49.8)	43.6 (38.6; 50.0)	0.878	40.1 (34.5; 43.5)	40.8 (36.5; 44.2)	0.645	40.5 (34.6; 52.2)	41.8 (38.1; 46.3)	0.950	36.1 (33.7; 47.6)	43.3 (37.4; 43.5)	1.000
PaO_2, mm Hg	125 (122; 142)	110 (94; 150)	0.574	132 (90; 146)	84 (76; 94)	**0.028**	114 (91; 132)	84 (70; 107)	0.081	106 (91; 125)	95 (78; 114)	0.530
BE, mmol/L	3 (–2; 8)	1 (–1; 3)	0.645	–2 (–6; 1)	–2 (–3; 0)	0.645	–2 (–7; 1)	–5 (–8; –1)	0.491	1 (–1; 1)	–4 (–9; 0)	0.073
HCO_3^-, mmol/L	27.5 (23.6; 32.3)	25.5 (24.1; 27.7)	0.574	21.6 (19.5; 25.6)	23.9 (21.8; 24.6)	0.328	23.5 (18.0; 27.1)	21.3 (18.6; 24.7)	0.573	26.2 (24.9; 27.0)	20.5 (14.6; 25.6)	0.106
Lactate, mmol/L	1.95 (0.95; 2.53)	1.91 (1.49; 2.91)	0.721	4.58 (2.87; 4.94)	6.10 (5.43; 7.71)	**0.005**	6.73 (5.25; 9.70)	6.35 (6.09; 8.09)	0.852	7.65 (3.42; 11.69)	6.02 (5.54; 6.34)	0.432
SaO_2, %	99 (98; 99)	98 (97; 100)	0.798	99 (97; 99)	96 (94; 97)	**0.015**	98 (95; 99)	96 (91; 98)	0.228	98 (96; 98)	97 (96; 98)	1.000
RBC, $\times10^{12}$/L	8.17 (7.53; 9.65)	8.05 (6.46; 10.0)	0.779	7.80 (7.09; 8.31)	7.25 (5.54; 8.26)	0.336	7.70 (7.21; 8.17)	7.00 (6.50; 7.96)	0.282	7.69 (6.72; 7.76)	7.63 (7.38; 8.12)	0.648
HGB, g/L	90 (81; 92)	87 (82; 93)	0.775	79 (73; 89)	75 (73; 90)	0.945	78 (71; 85)	90 (79; 95)	0.240	72 (71; 81)	96 (83; 102)	0.111
HCT, %	33.5 (28.2; 35.3)	32.5 (30.0; 38.7)	0.867	30.4 (25.8; 34.5)	29.2 (26.2; 33.6)	0.955	31.0 (27.4; 34.6)	32.8 (28.0; 34.4)	0.662	28.7 (27.7; 35.3)	34.8 (30.6; 36.9)	0.230
PLT, $\times10^9$/L	779 (719; 796)	682 (618; 835)	0.463	683 (646; 739)	641 (582; 808)	0.955	662 (621; 760)	636 (432; 740)	0.573	668 (556; 819)	716 (570; 805)	0.927

Note: HCO_3^- — bicarbonate concentration; HCT — hematocrit; HGB — hemoglobin level; PLT — thrombocyte level; RBC — total erythrocyte level; SaO_2 — arterial blood saturation; BE — base deficit; PaO_2 — partial pressure of oxygen of arterial blood; $PaCO_2$ — partial pressure of arterial carbon dioxide; pH — negative logarithm of hydrogen ions concentration; BV — blood volume.

(Table 1). Statistical analysis did not show significant differences in these findings between groups ($p > 0.05$).

Discussion

Acute massive blood loss remains one of the most urgent challenges in the critical care medicine, posing a threat to the patient life [19–22]. Special attention should be given to the situations associated with long-time patients' evacuation to the hospital or patients' evacuation from active combat zone, when time period to providing competent medical care can significantly exceed critical time limits instituted by the rule of "golden hour". According to estimates, about half of the patients with AMBL die before reaching a hospital [23, 24], therefore pre-hospital care is critical to early detection and adequate treatment of blood loss [25]. At present the search for techniques capable to extend the survival period and stabilize the AMBL patient's condition before reaching specialized medical facility remains important scientific and practical problem to be solved.

The received data on survival rate increase in the experimental group, more stable hemodynamics and less pronounced changes in the gas and acid-base arterial blood composition in the setting of gas mixture (argon and krypton) inhalation in the simulation of AMBL without infusion-based resuscitation suggest that the key cytoprotective effect occurs at the level of ischemic damage cell protection. The following hypotheses for molecular mechanisms of these processes can be offered:

- **Apoptosis suppression.** The main cytoprotective effect of noble gases can be associated with intracellular signaling pathway activation (such as PI3K/Akt, ERK1/2, increase in anti-apoptotic protein Bcl-2 level, decrease in pro-apoptotic protein Bax level etc.), that inhibit mitochondrial apoptotic pathway [26–28]. Argon exhibits cardioprotective properties which presumably are realized owing to increase in microRNA-21 expression level which in its turn inhibit pro-apoptotic protein synthesis PDCD4 and PTEN in cardiomyocytes [29, 30]. This promotes longer-term maintenance of viable cells in vital organs (myocardium, brain, kidneys) in the setting of deep ischemia.
- **Inflammatory response modulation.** Argon and especially krypton may affect the activity of key pro-inflammatory transcription factors (NF-κB), decreasing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6]. It could have a limiting effect on the development of systemic inflammatory response syndrome, aggravating multiple organ dysfunction in the presence of shock [31].

In the study of AMBL simulation survival rate appeared to be much higher in animals receiving respiratory gas mixture with elevated noble gas concentration compared with the group receiving oxygen-air mixture. MAP plays a major role in the assessment of adequate vital organs blood supply, e.g. heart, brain, kidneys. Low levels of MAP are indicative of insufficient tissue perfusion, that can result in organ failure. To compensate AMBL in the presence of BV decrease in animals, compensatory tachycardia develops. During the experiment more stable findings of MAP (at the end of blood loss) and HR (during monitoring) were recorded in the group receiving respiratory mixture with increased noble gases concentration compared to oxygen-air mixture. This difference in the hemodynamic findings in the setting of equal blood loss volume in animals may be due to specific systemic response to hypovolemic changes. The noble gases studied possess cytoprotective properties, krypton has additionally anti-stress effect, which can promote less pronounced sympathoadrenal response and pathological vasoconstriction. Under these circumstances more effective MAP maintenance may occur thanks to more rational distribution of vasomotor tone and improved microcirculation maintenance, not only because of compensatory tachycardia. Moreover, MAP stabilization may be associated with endothelium protective properties of noble gas caused by general reduction in oxidative stress which potentially promotes improvement of organs and tissues microperfusion.

When using gas mixture with argon and krypton one could note less pronounced acid-base balance changes by the end of monitoring, improved arterial blood gas composition (PaO_2, SaO_2) and much more gradual increase in lactate level after AMBL. When AMBL occurs in the setting of hypovolemia, metabolic acidosis develops which is due to BV decrease and tissue oxygen supply deficit. Because of this, anaerobic metabolism activation occurs, that is accompanied by lactate accumulation. In case of AMBL arterial PaO_2 decrease occurs because of acute and significant BV decrease and, as a consequence, cardiac output reduction, pulmonary perfusion and gas exchange impairment. Since respiratory support was delivered with minimal adjustment of ventilator parameters during the experiment, changes in PaO_2 reflected the effects of AMBL and its complications. At the same time SaO_2 decrease occurred due to oxygen transport function deficiency, pulmonary function impairment and oxyhemoglobin dissociation curve bias to the right caused by tissue hypoxia. Less pronounced metabolic acidosis and better maintaining blood gas composition indicate more effective aerobic metabolism in tissues of animals of the experimental group. This is a direct consequence of anticipated cytoprotective effect of the mixture — maintenance of mitochondrion function in the presence of ischemia provides maintenance of oxidative phosphorylation in cells, reducing dependence on anaerobic glycolysis, as well as lactic acid accumulation.

Study limitations

It is highly likely that the absence of statistical differences in the traditional analysis of survival was associated with animals small sample in the experiment, because of this Moses test, where extreme outliers are cut off, demonstrated the presence of statistical differences.

Conclusion

The present study showed that the use of respiratory mixture with elevated noble gases content (60 % argon, 30 % oxygen and 10 % krypton) promotes survival growth of large laboratory animals in acute massive blood loss simulation. This effect was accompanied by oxygenation enhancement and improvement of acid-base balance, that is indicative of effectiveness of the mixture in maintaining compensatory body mechanisms in case of the development of metabolic disturbances. The study results allow to consider the use of respiratory mixture with elevated noble gas level to be a prospective method of patient's condition stabilization and prevention of acute massive blood loss complications, especially in the setting of delayed medical care or prolonged transportation.

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Author contribution. All authors according to the ICMJE criteria participated in the development of the concept of the article, obtaining and analyzing factual data, writing and editing the text of the article, checking and approving the text of the article.

Ethics approval. This study was approved by the local independent Ethical Committee at the S.M. Kirov Military Medical Academy (reference number: 279-27.06.2023).

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