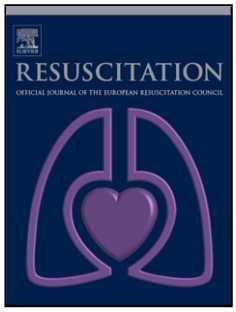

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Authors: Giovanni Babini, Giuseppe Ristagno, Antonio Boccardo, Daria De Giorgio, Marcella De Maglie, Roberta Affatato, Sabina Ceriani, Davide Zani, Deborah Novelli, Lidia Staszewsky, Serge Masson, Davide Pravettoni, Roberto Latini, Angelo Belloli, Eugenio Scanziani, Markus Skrifvars

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Effect of mild hypercapnia on outcome and histological injury in a porcine post cardiac arrest model

Authors:

Giovanni Babini (giovanni.babini@guest.marionegri.it),[1,2]

Giuseppe Ristagno (gristag@gmail.com),[1]

Antonio Boccardo (antonio.boccardo@unimi.it),[3]

Daria De Giorgio (daria.degiorgio@marionegri.it),[1]

Marcella De Maglie (marcella.demaglie@unimi.it),[3,4]

Roberta Affatato (roberta.affatato@marionegri.it),[1]

Sabina Ceriani (sabina.ceriani@gmail.com),[3]

Davide Zani (davide.zani@unimi.it),[3]

Deborah Novelli (deborah.novelli@marionegri.it),[1]

Lidia Staszewsky (lidia.staszewsky@marionegri.it),[1]

Serge Masson (serge.masson@marionegri.it),[1]

Davide Pravettoni (davide.pravettoni@unimi.it),[3]

Roberto Latini (roberto.latini@marionegri.it),[1]

Angelo Belloli (angelo.belloli@unimi.it),[3]

Eugenio Scanziani (eugenio.scanziani@unimi.it),[3,4]

Markus Skrifvars (markus.skrifvars@hus.fi).[5,6]

Affiliations:

[1]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy

[2]Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, University of Milan, Italy

[3]Dipartimento di Medicina Veterinaria, University of Milan, Lodi, Italy

[4]Mouse and Animal Pathology Lab (MAPLab), Fondazione Filarete, Milan, Italy

[5]Division of Intensive Care, Department of Anaesthesiology, Intensive Care and Pain Medicine, Helsinki University Hospital and University of Helsinki, Finland

[6]Emergency Medicine and Services, Helsinki University Hospital and Department of Emergency Medicine, University of Helsinki, Finland

Corresponding author

Markus Skrifvars, MD, PhD, EDIC, FCICM

Haartmaninkatu 4

00029 HUS

Finland

markus.skrifvars@hus.fi

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Abstract

Aim of the study. To evaluate in an established porcine post cardiac arrest model the effect of a mild hypercapnic ventilatory strategy on outcome.

Methods. The left anterior descending coronary artery was occluded in 14 pigs and ventricular fibrillation induced and left untreated for 12 min. Cardiopulmonary resuscitation was performed for 5 min prior to defibrillation. After resuscitation, pigs were assigned to either normocapnic (end-tidal carbon dioxide ($EtCO_2$) target: 35-40 mmHg) or hypercapnic ventilation ($EtCO_2$ 45-50 mmHg). Hemodynamics was invasively measured and $EtCO_2$ was monitored with an infrared capnometer. Blood gas analysis, serum neuron-specific enolase (NSE) and high sensitive cardiac troponin T (hs-cTnT) were assessed. Survival and functional recovery were evaluated up to 96 h.

Results. Twelve pigs were successfully resuscitated and eight survived up to 96 h, with animals in the hypercapnic group showing trend towards a longer survival. $EtCO_2$ and arterial partial pressure of CO_2 were higher in the hypercapnic group compared to the normocapnic one ($p<0.01$), during the 4-hour intervention. Hypercapnia was associated with higher mean arterial pressure compared to normocapnia ($p<0.05$). No significant differences were observed in hs-cTnT and in NSE between groups, although the values tended to be lower in the hypercapnic one. Neuronal degeneration was lesser in the frontal cortex of hypercapnic animals compared to the normocapnic ones ($p<0.05$). Neurological recovery was equivalent in the two groups.

Conclusion. Mild hypercapnia after resuscitation was associated with better arterial pressure and lesser neuronal degeneration in this model. Nevertheless, no corresponding improvements in neurological recovery were observed.

Protocol no. 84/2014-PR

Introduction

Despite of continuous improvements in post-resuscitation (PR) care, outcome of cardiac arrest (CA) remains poor. Indeed, more than half of resuscitated patients die early after hospital admission,[1,2] such that the final survival ranges from 0.5 to 20% in Europe, with great differences among countries.[3,4] For this reason, one of the main objective of resuscitation science is investigating novel therapeutic strategies that may improve survival with good neurological recovery.[5,6] For example, the optimal respiratory targets, i.e. ideal blood partial pressure (Pa) of oxygen (O_2) and carbon dioxide (CO_2) are currently unknown and several specific trials have been undertaken.[7-9]

Recently, the role of $PaCO_2$ became of particular interest after the publication of a series of experimental and observational studies anticipating potential benefits on CA outcome from a ventilation pattern set to maintain a mild hypercapnia.[8,10,11] In a rodent model of global cerebral ischemia but without cardiac arrest, mild to moderate hypercapnia ($PaCO_2$ 60–100 mmHg) was associated with lesser histological brain damage and lesser apoptosis compared to normocapnia or severe hypercapnia after cerebral ischemia.[12] Pathophysiologically, hypercapnia holds interesting properties that might be beneficial for CA patients such as an increase in cerebral blood flow, an increase in arterial blood pressure due to endogenous catecholamine release, and an attenuation of ischemia-reperfusion injury due to mitigation of oxidative stress.[13-15]

This study aimed to investigate the effects of PR mild hypercapnia on survival and functional recovery in a porcine post-CA model. We hypothesized that a period of hypercapnia after resuscitation might ameliorate cardiac and neurological dysfunction and survival compared to normocapnia.

Material and methods

All procedures involving animals and their care were in conformity with national and international laws and policies. Approval of the study was obtained by the governmental review board, within a wider protocol on inhalatory strategies to improve CA outcome (N.84/2014-PR).

Animal Preparation

Fourteen male domestic pigs (38±3 kg) were fasted the night before experiment except for free water access. Anaesthesia was induced by intramuscular injection of ketamine (20 mg/kg) followed by intravenous administration of propofol (2 mg/kg) and sufentanyl (0.3 μg/kg) through an ear vein access. Anaesthesia was then maintained by continuous intravenous infusion of propofol (4-8 mg/kg/h) and sufentanyl (0.3 μg/kg/h). Intravenous cis-atracurim (0.5 mg/kg) was administered at hourly interval for muscle relaxation. A cuffed tracheal tube was placed, and animals were mechanically ventilated with a tidal volume of 15 mL/kg and fraction of inspired oxygen (FiO_2) of 0.21. During the preparation phase, respiratory frequency was adjusted to maintain the end-tidal partial pressure of carbon dioxide ($EtCO_2$) between 35-40 mmHg, monitored with an infrared capnometer.[16] For measurement of aortic pressure, a fluid-filled 7F catheter was advanced from the right femoral artery into the thoracic aorta. For measurements of right atrial pressure (RAP), core temperature, and cardiac output (CO), a 7F pentalumen thermodilution catheter was advanced from the right femoral vein into the pulmonary artery. Myocardial infarction was induced in a closed-chest preparation by intraluminal occlusion of the left anterior descending (LAD) coronary artery with the aid of a 6F balloon-tipped catheter inserted from the right common carotid artery.[16] For inducing ventricular fibrillation (VF), a 5F pacing catheter was advanced from the right jugular vein into the right ventricle. The position of all catheters was confirmed by characteristic pressure morphology and/or fluoroscopy. Frontal plane electrocardiogram was recorded.

Experimental procedure

Fifteen min prior to induce CA, animals were randomized into hypercapnia or normocapnia ventilation. The balloon of the LAD coronary artery catheter was then inflated with 0.7 mL of air to occlude the flow. If VF did not occur spontaneously, after 10 min it was induced with 1-2 mA AC current delivered to the right ventricle endocardium.[16] Ventilation was discontinued after onset of

VF. After 12 min of untreated VF, cardiopulmonary resuscitation (CPR), including chest compressions with the LUCAS 2 (PhysioControl Inc.) and ventilation (tidal volume of 500 mL, 10 breaths/min, FiO2 100%), was initiated. After 5 min of CPR, defibrillation was attempted with a single biphasic 150-J shock, using an MRx defibrillator (Philips Medical Systems). If resuscitation was not achieved, CPR was resumed and continued for 1 min prior to a subsequent defibrillation. Adrenaline (30 µg/kg) was administered via the right atrium after 2 and 7 min of CPR. Successful resuscitation was defined as restoration of an organized cardiac rhythm with a mean arterial pressure (MAP) of more than 60 mmHg. After that, if VF reoccurred, it was treated by immediate defibrillation. After successful resuscitation, anaesthesia was maintained, and animals were monitored during the 4-h treatment.

Animals were subjected to a 4-h normocapnia or hypercapnia ventilation. For this aim, respiratory rate was adjusted while keeping tidal volume constant, in order to obtain an EtCO2 within 35-40 mmHg in the control normocapnic group or within 45-50 mmHg in the treatment hypercapnic group. Forty-five minutes after resuscitation, the LAD coronary artery catheter was withdrawn. Temperature of the animals was maintained at 38±0.5°C during the whole experiment. After 4 h of treatment, catheters were removed, wounds were repaired, and the animals were extubated and returned to their cages. Analgesia with butorphanol (0.1 mg/kg) was administered by intramuscular injection. At the end of the 96 h PR period, animals were sedated with intramuscular ketamine (20 mg/kg) and intravenous propofol (1 mg/kg bolus, followed by 2-3 mg/kg/h infusion) through an ear vein access and maintained spontaneously breathing for echocardiographic examination and blood sample withdrawn by direct femoral artery puncture. Animals were then sacrified painlessly with an intravenous injection of 150 mg/kg sodium thiopental, and heart and brain were harvested. Autopsy was performed routinely for potential injuries due to CPR or obfuscating disease. The experimental design is summarized in Figure 1.

Measurements

Hemodynamics, $EtCO_2$, and electrocardiogram were recorded continuously on a personal computer-based acquisition system (WinDaq DATAQ Instruments Inc). The coronary perfusion pressure was computed from the differences in time-coincident diastolic aortic pressure and RAP. CO was measured by thermodilution technique (COM-2; Baxter International Inc). Transthoracic echocardiography was performed using a phase-array multifrequency 2.5-5-MHz probe (CX50, Philips, Spa). Two-dimensional apical four chamber view was acquired to determine left ventricular (LV) volumes and ejection fraction (EF); calculations were computed using the modified single-plane Simpson's rule.[16] Stroke volume was calculated by applying the formula: CO/heart rate. CO was determined as the product of the time-velocity integral of the outflow curves obtained in 5-CH apical view using pulsed wave Doppler and the cross sectional area of the aortic anulus obtained from 2D-echocardiography image in parasternal long-axis view. Arterial blood gases were assessed with i-STAT System (Abbott Laboratories). Plasma high-sensitivity cardiac troponin T (hs-cTnT) and serum neuron-specific enolase (NSE) were measured with electrochemiluminescence assays (Roche Diagnostics Spa).

As previously described,[16] neurologic recovery was assessed with the neurologic alertness score (NAS), ranging from 100 (normal) to 0 (brain death), and with the swine neurologic deficit score (NDS), ranging from 0 (normal) and 400 (brain death). Finally, the functional recovery was evaluated prior to sacrifice according to overall performance categories (OPC), ranging from 1=normal to 5=brain death or death.[16] Outcome was defined poor when OPC was ≥3. Scores were assessed by veterinary doctors blinded to treatment.

At sacrifice, the brains were carefully removed from the skulls and fixed in 4% buffered formalin. Standardized 5-mm coronal slices were taken. The hippocampal CA1 sector and the cortex were chosen as regions of interest and were paraffin embedded. Five-micrometer-thick sections were then obtained and stained with hematoxylin-eosin. The proportion of neuronal loss and

degeneration/necrosis (shrunken neurons with deeply acidophilic cytoplasm and pyknotic nucleus) was quantified as absent (0), rare (1), few (2), and numerous (3). For apoptosis, sections were stained immunohistochemically with a rabbit polyclonal anti-cleaved caspase-3 primary antibody. For evaluation of vascular leakage, as expression of endothelial cell damage in capillary ultrastructure, [17] sections were stained with a rabbit polyclonal anti-albumin antibody. Details in Supplementary Figure 1. An experienced pathologist, blinded to treatment, performed the assessments.

Myocardial infarct was assessed by tetrazolium chloride (TTC) staining in 5-mm-thick LV transverse sections. Infarct size was reported as percentage of TTC-negative area relative to LV area.[16]

Statistical Analysis

One sample Kolmogorov–Smirnov Z test was used to confirm normal distribution of the data. For comparisons of time-based variables, repeated measures analysis of variance (ANOVA) with Holm-Sidak's multiple comparison was used. For comparisons between groups at the given time points, one-way ANOVA was used for normally distributed variables, while Kruskal-Wallis test with Dunn's multiple comparison was used for not normally distributed variables. When the dependent variable was categorical, χ^2 test was performed. For survival analysis, Kaplan-Meier survival curves and log-rank (Mantel-Cox) test were used. Data are expressed as mean ± standard deviation (SD), except for hs-cTnT and NSE, presented as median and interquartile range (IQR). The sample size was estimated regarding the neurological functional score NAS [5]. To show a NAS recovery in the hypercapnia group, i.e. a 45% NAS increase compared to control, 7 pigs per group were needed (β 0.8, α 0.05). A $p \leq 0.05$ was regarded as statistically significant. Data analyses were performed using GraphPad Prism (version 6.05 for Windows, GraphPad Software, USA).

Results

No significant differences in body weight, hemodynamics, myocardial function, and blood gas analyses were observed between groups at baseline (Table). All the animals, except 2 in the hypercapnia group, were successfully resuscitated. The duration of CPR and the total number of defibrillation delivered were similar in both groups (Table). Eighty percent of the animals resuscitated from CA and treated with hypercapnia survived till 96 h compared to 57% in the normocapnia group (Figure 2).

After resuscitation, $EtCO_2$ and $PaCO_2$ were significantly higher in the hypercapnia group compared to the normocapnia one, during the 4 h of treatment (Table). Animals in the hypercapnia group showed a trend toward a lower arterial pH and PaO_2 compared to normocapnia ones, although the differences were not significant (Table). The hypercapnia group showed a significantly higher PR systolic, mean, and diastolic arterial pressures compared to the normocapina one ($p<0.05$, Figure 3). No effects of hypercapnia on heart rate and RAP were observed (Table).

PR myocardial function was depressed in all animals. No differences in EF and stroke volume, between the two groups were observed during the whole period of observation (Table). Hypercapnic animals showed a trend toward smaller LV end-diastolic and end-systolic volumes compared to hypercapnic ones during the first 4 h of treatment.

No difference in LV infarct size was observed in the two groups (Table) and this result was concordant with the plasma levels of hs-cTnT. Only a trend towards a lower hs-cTnT in the hypercapnia group was reported at 96 h after resuscitation (56 vs. 153 ng/L, p not significant; Figure 4).

A good neurological recovery, i.e. OPC 1-2, was observed in 20% of the animals in the hypercapnia group and in 29% in the normocapnia one (p not significant, Table). No significant differences were

also observed in the NAS and NDS scores in the group treated with hypercapnia compared to normocapnia(Figure 5). Overall none or only a few apoptotic neurons and interstitial vascular leakage were observed in the cerebral cortex (caspase-3 staining (score): 0.5±0.6 in hypercapnia vs. 0.5±1 in control; albumin staining (score): 0.5±0.6 in hypercapnia vs. 0.3±0.5 in control; p not significant) and CA1 hippocampal sector (caspase-3: 0.75±0.5 in hypercapnia vs. 0.75±1.5 in control; albumin: 0.75±0.5 in hypercapnia vs. 0.5±1 in control; p not significant) of all the resuscitated animals. Only one animal in the control group showed extensive apoptosis (2+ in cortex and 3+ in CA1) and vascular leakage (2+ in cortex) (Supplementary Figure 1). Nevertheless, a significant lesser severe neuronal degeneration in the frontal cortex of animals treated with hypercapnia in comparison to those in normocapnia was observed (p=0.03, Figure 5). This result was supported by a lower 96 h serum NSE concentration in the hypercapnia group (14 vs 21 ng/mL, p not significant; Figure 4). No differences in neurological degeneration in the hippocampal CA1 sector were observed.

Discussion

The present study investigated the effect of post resuscitation mild hypercapnia, obtained by reducing minute ventilation, as a potential treatment to improve outcome of CA. Indeed, in our model, a 4-h period of mild hypercapnia was associated with a better mean arterial pressure and a decrease in neuronal degeneration in the frontal cortex; nevertheless, no corresponding functional improvements in neurological recovery were observed.

CO_2 is the major determinant of cerebral blood flow, with high $PaCO_2$ causing vasodilatation and increased brain perfusion, whereas low $PaCO_2$ induces vasoconstriction and reduced cerebral blood flow.[13] It has been suggested that vascular reactivity to CO_2 is blunted in the immediate PR period, but some evidences suggest that it is preserved after ROSC [18-22]. Thus, mild to moderate hypercapnia could alleviate ischemia, especially in the setting of detrimental cerebral vasoconstriction, which appears to be the case in post CA patients.[23] Our study confirmed a lesser

neuronal injury in the frontal cortex in animals treated with mild hypercapnia, compared to normocapnia. We did observe a trend towards lower serum NSE release and frontal lobe neuronal injury, however, no differences regarding the degree of neuronal degeneration in hippocampal CA1 sector and the neurological scores were observed. It is possible that some areas suffered more from hypoperfusion and hypoxia despite a global increase in cerebral blood flow and in oxygen delivery.

Carbon dioxide has also been suggested to exert neuroprotective effects independently of any modifications in cerebral perfusion.[12] For example, hypercapnic acidosis may attenuate the production of superoxide catalysed by xanthine oxidase, decreasing neuronal oxidative stress.[24] Moreover, high level of $PaCO_2$ may reduce the levels of detrimental amino acids like glutamate, thus reducing the excitotoxicity induced by high level of excitatory neurotransmitters.[25] We did not perform any analysis on molecular pathways of neuronal injury and any possible interaction with CO2, so we can only speculate about any direct effect of hypercapnia on neuroprotection.

We observed a consistently higher arterial blood pressure during the 4-h treatment in the hypercapnic animals compared to the normocapnic ones. This might have been related to an increase in the sympathetic tone due by the release of endogenous catecholamines into the systemic circulation.[25,26] This effect might have been, however, counterbalanced by a direct cardiovascular depressant effect related to the acidosis with blunting of the catecholamine sensitivity.[27,28] The hypercapnia-induced increase in diastolic arterial pressure observed in the current study could have theoretically resulted in higher coronary perfusion pressure, but this did not translate into a better myocardial function or a decrease in myocardial injury. The slightly lower systemic pH might have increased myocardial oxygen consumption due to increases in left and right ventricle afterload and therefore blunted any hypercapnia-related beneficial effect.[25,29]

Hypercapnia may also have deleterious effects such as an increase in intracranial pressure.[30] Whether the concomitant increase in MAP results in greater cerebral perfusion pressure maintaining adequate global cerebral perfusion pressure is currently unclear.[31,32] In our study we did not

monitor intracranial pressures, so we cannot comment on whether there was any difference in cerebral perfusion pressure between groups. Nevertheless, none or only a slight leakage of serum albumin to the cerebral interstitial space was observed in hypercapnic animals, thus potentially excluding hypercapnia-related development of brain edema.[17]

In humans, a possible beneficial effect of mild hypercapnia has been shown with data from observational studies.[10,11,19] In the only randomized controlled trial, compared to normocapnia (35-45 mmHg), mild hypercapnia (50–55 mmHg) was associated with significantly lower NSE, while no adverse effects related to higher PaCO2 were reported.[8] A large randomized controlled trial is planned on the effect of moderate hypercapnia on neurological outcome after out-of-hospital CA.[33]

We recognize limitations in the interpretations of our findings. Firstly, we only tested two different levels of hypercapnia for a period of 4 h and during normothermia. Secondly, we focused on neurological functional outcome, yet neglecting underlying potential mechanisms of action of PR hypercapnia. The 96-h histopathology was probably a too late assessment to show direct effects of hypercapnia on the evolving brain injury. Indeed, since none or only a few apoptotic neurons were observed in both groups, it cannot be excluded that neurons had already passed through the execution phase of apoptosis. Indeed, experimental models have shown caspase-3 activity to peak within 72 h after ischemia, while a marked decrease was observed by 96 h, in favor of severe degenerative changes.[34, 35] Thirdly, the direct effects of oxygen on CA outcome were not studied directly in the present study; however, oxygen may have influenced the physiological effects of CO_2, as previously reported.[10,36,37] Indeed, in a prospective observational study hypercapnia was associated with good neurological outcome at 12 month after resuscitation and this effect was clearly influenced by oxygen blood tension: the combination of moderate hypercapnia and mild hyperoxia was associated with improved neurological outcome.[10] In our study we observed a trend towards a lower PaO_2 in the hypercapnia group, due to the low minute ventilation, which might have aggravated the systemic acidosis and hidden potential benefits from hypercapnia.[38] Nevertheless, PaO_2 was still in the

physiological range, while the hypercapnia-related mild acidosis is known to determine a rightward shift of the haemoglobin dissociation curve, facilitating tissue O_2 release.[15] Most likely, in our model, animals were excessively hypoventilated causing atelectasis, and consequently hypoxemia, as also suggested by the high alveolar-arterial $PaCO_2$ difference.[39-40] Thus "therapeutic hypercapnia" may require the use of a higher positive end expiratory pressure or a higher fraction of inspired oxygen to avoid hypoxaemia. Indeed, the Carbon dioxide, Oxygen, and Mean arterial pressure After Cardiac Arrest and REsuscitation (COMACARE) trial (NCT02698917) is ongoing, which assess the feasibility of targeting low- or high-normal $PaCO_2$, PaO_2, and MAP in PR comatose, mechanically ventilated patients, as well as its effect on brain injury markers.

Conclusions

Our study partially confirmed the hypothesis of a beneficial effects of mild hypercapnia on CA outcome with better mean arterial pressure and a lesser neuronal degeneration in the frontal cortex. However, no corresponding improvements in neurological recovery or survival was observed. A greater acidotic status and a lower PaO_2 observed during hypercapnia might have blunted its cardio- and neuroprotective effects.

Conflicts of interest

All authors declare no conflicts.

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Figure legends

Figure 1. Schematic representation of the experimental design. VF, ventricular fibrillation; CPR, cardiopulmonary resuscitation; EKG, electrocardiography; EF, ejection fraction; P art, arterial pressure; P r.atrium, right atrial pressure; ECHO, echocardiography; LAD, left anterior descendent coronay artery; ROSC, return of spontaneous circulation; $EtCO_2$, End-tidal CO_2 partial pressure; min, minutes; h, hours.

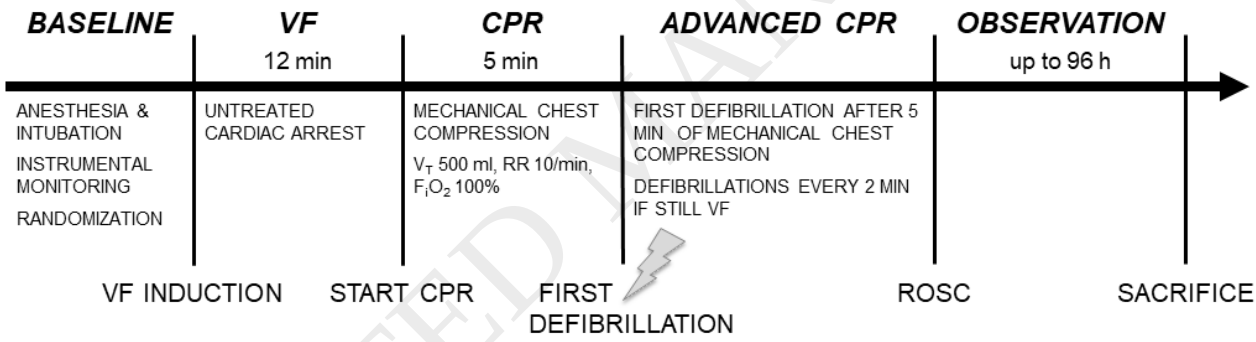

Figure 2. Kaplan–Meier curves of post-resuscitation survival up to 96 h in animals successfully resuscitated after CA. Survival comparison was performed by log-rank (Mantel-Cox) test. In brackets, the number of animals alive throughout the observational period.

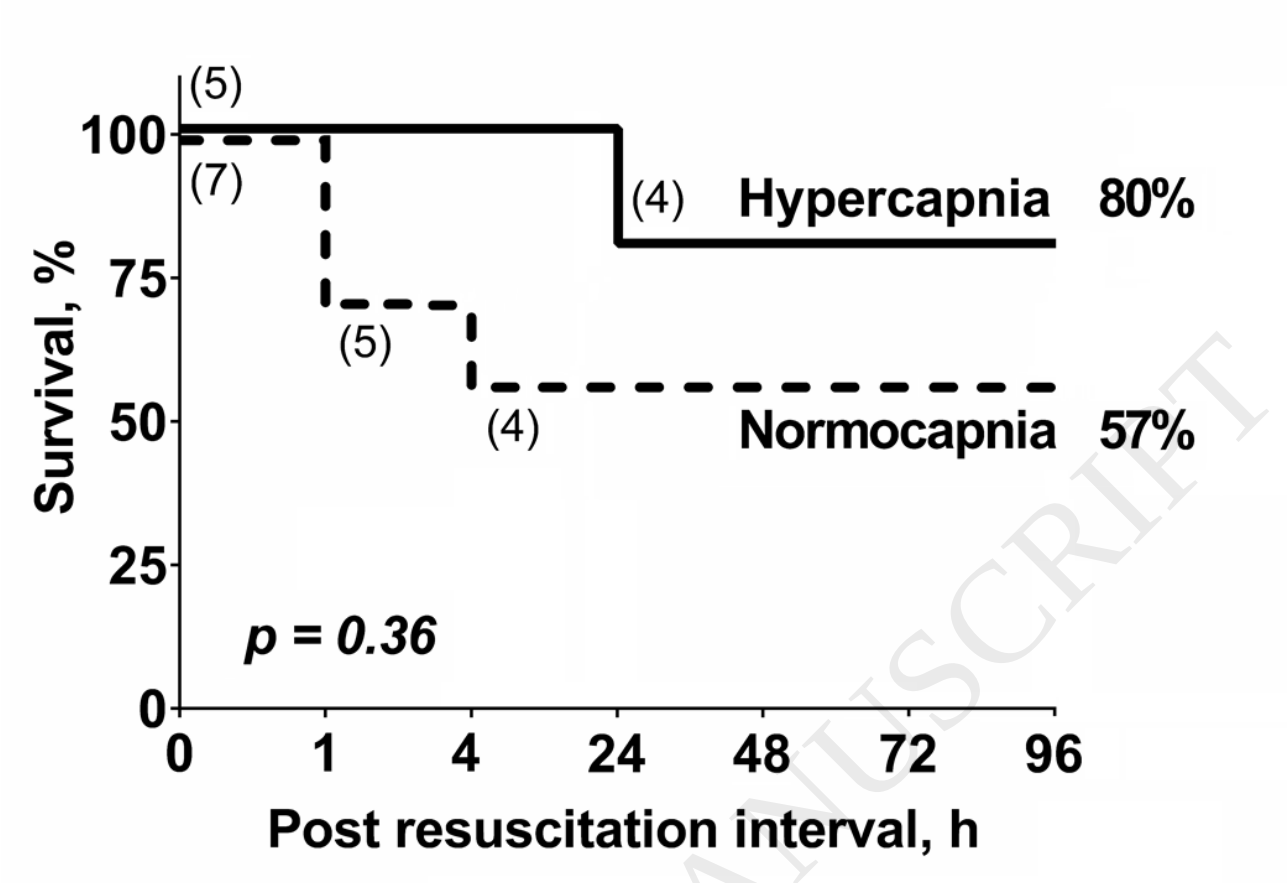

Figure 3. Mean arterial pressure (MAP), systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) at baseline and during the first 4 h post resuscitation. Data are shown as mean ± SD. * $p < 0.05$, repeated measures ANOVA with Holm-Sidak's multiple comparison.

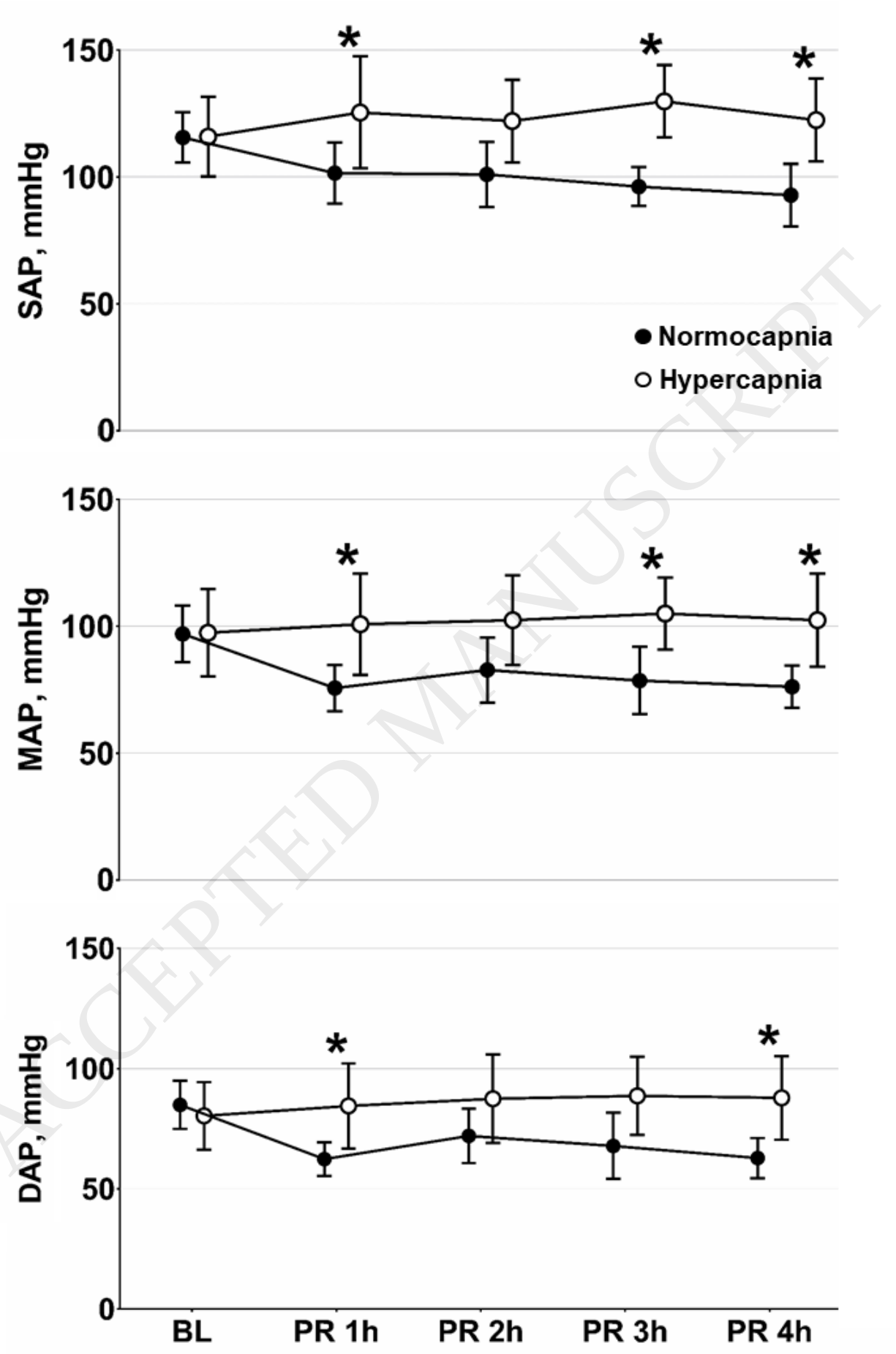

Figure 4. A. Plasma high sensitive cardiac troponin T (hs-cTnT) at baseline, 2 h, 4 h and 96 h post resuscitation. B. Serum neuronal specific enolase (NSE) at baseline and 96 h post resuscitation.

Data are expressed as median and interquartile range. Kruskal-Wallis test with Dunn's multiple comparison was used to compare the groups.

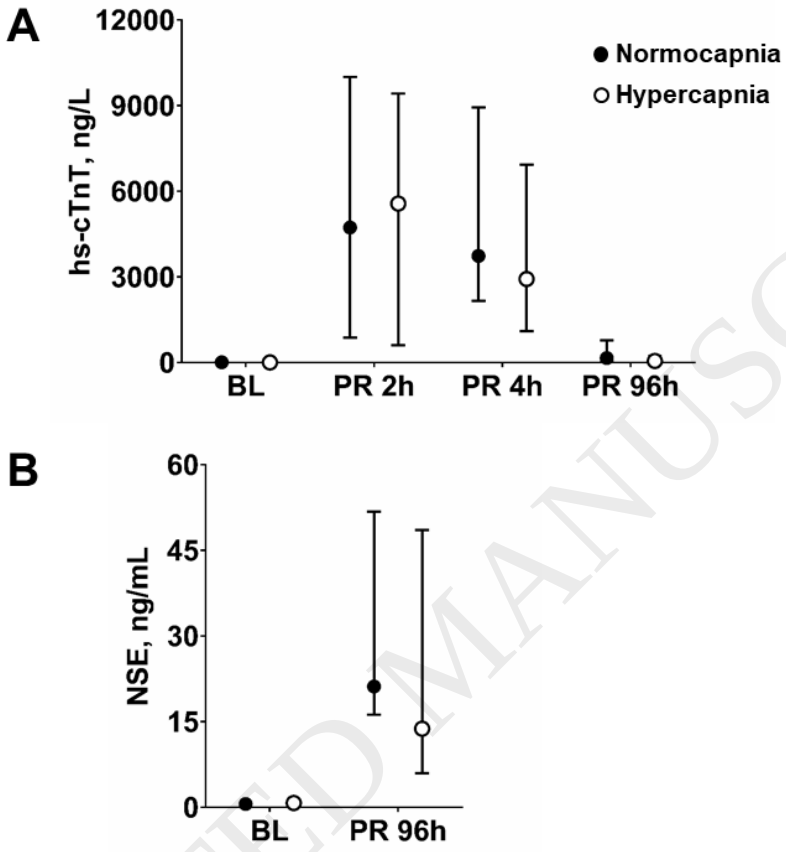

Figure 5. A. Overall performance category (OPC). χ^2 test was used for comparison. Data are expressed as individual data point and mean of the group (OPC ≤ 2 was considered as good neurological outcome). B. Neuronal degeneration in CA1 hippocampal sector and frontal cortex (one-way ANOVA). C. Neurologic alertness score (NAS) and D. Neurological deficit score (NDS) evaluated in animals successfully resuscitated from cardiac arrest at 24, 48 and 72 h post resuscitation (repeated measures ANOVA with Holm-Sidak's multiple comparison).

Data are expressed as mean ± SD. * $p < 0.05$.

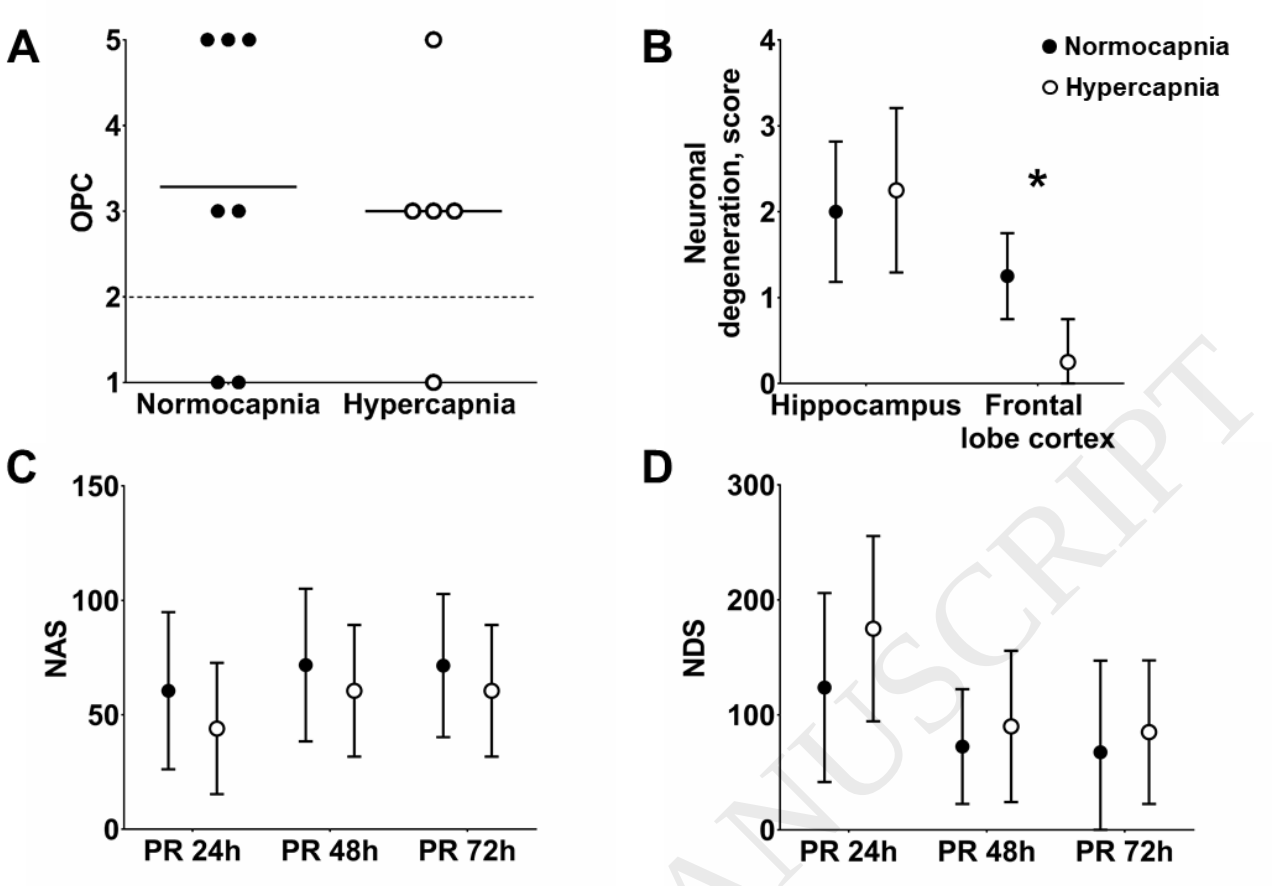

Table 1. Resuscitation outcomes, hemodynamics, echocardiographic data and blood, gas analysis

	Normocapnia (n=7)	**Hypercapnia** (n=7)	**p value**
Successful resuscitation, n/n (%)	7/7 (100)	5/7 (71)	0.13
Total defibrillations, n	18 ± 18	14 ± 9	0.68
Duration of CPR, sec	394 ± 189	438 ± 234	0.73
Survival duration, h	56 ± 50	81 ± 34	0.36
96 h survival, n/n (%)	4/7 (57)	4/5 (80)	0.36
OPC ≤ 2, n/n (%)	2/7 (29)	1/5 (20)	0.79
Heart Rate, beat/min			
BL	103 ± 28	113 ± 44	0.65
PR 2h	171 ± 23	162 ± 51	0.74
PR 4h	153 ± 43	177 ± 30	0.33
Right Atrial Pressure, mmHg			
BL	6 ± 2	5 ± 2	0.31
PR 2h	8 ± 2	6 ± 2	0.11
PR 4h	8 ± 1	7 ± 4	0.78
End-Tidal CO_2, mmHg			
BL	37 ± 2	38 ± 2	0.27
PR 2h	36 ± 1	48 ± 1	< 0.01
PR 4h	36 ± 2	48 ± 2	< 0.01
LV CO, L/min			
BL	4.5 ± 0.8	5.0 ± 1.7	0.52
PR 2h	3.3 ± 0.9	3.9 ± 0.6	0.27
PR 4h	2.8 ± 0.5	2.7 ± 1.3	0.87

LV infarct size area, %	9.0 ± 4.4	6.3 ± 6.5	0.52
LV end-diastolic volume, ml			
BL	36.6 ± 9.7	30.6 ± 1.8	0.209
PR 2h	38.0 ± 7.2	30.9 ± 10.1	0.290
PR 4h	45.8 ± 16.3	28.1 ± 9.0	0.105
PR 96h	39.4 ± 14.8	41.6 ± 10.1	0.840
LV end-systolic volume, ml			
BL	8.5 ± 3.1	10.5 ± 2.8	0.360
PR 2h	24.0 ± 6.3	19.4 ± 7.4	0.382
PR 4h	28.5 ± 12.0	14.8 ± 8.9	0.117
PR 96h	14.0 ± 4.2	21.2 ± 11.6	0.291
LV ejection fraction, %			
BL	75.9 ± 8.7	65.5 ± 7.6	0.100
PR 2h	36.9 ± 8.3	37.9 ± 7.6	0.872
PR 4h	39.3 ± 9.2	51.2 ± 15.5	0.236
PR 96h	62.5 ± 6.6	49.0 ± 15.5	0.160
LV stroke volume, ml			
BL	42.2 ± 15.1	50.4 ± 6.2	0.307
PR 2h	22.4 ± 4.1	21.6 ± 4.1	0.788
PR 4h	22.2 ± 3.4	23.1 ± 9.8	0.883
PR 96h	34.4 ± 3.6	36.4 ± 11.5	0.828
pH			
BL	7.48 ± 0.08	7.46 ± 0.06	0.61
PR 2h	7.34 ± 0.07	7.23 ± 0.10	0.09
PR 4h	7.40 ± 0.08	7.27 ± 0.11	0.07

PaO_2, mmHg			
BL	76 ± 16	81 ± 9	0.46
PR 2h	111 ± 19	85 ± 24	0.09
PR 4h	103 ± 28	95 ± 21	0.62
$PaCO_2$, mmHg			
BL	38 ± 2	38 ± 3	0.60
PR2h	40 ± 2	54 ± 6	< 0.01
PR4h	41 ± 1	53 ± 7	< 0.01
HCO_3, mmol/L			
BL	29 ± 5	28 ± 2	0.64
PR2h	22 ± 4	23 ± 3	0.72
PR4h	25 ± 4	27 ± 5	0.65
BE, mmol/L			
BL	5 ± 6	4 ± 3	0.65
PR2h	- 3 ± 6	-5 ± 5	0.68
PR4h	1 ± 5	0 ± 6	0.83

For comparisons of resuscitation and survival outcomes, including OPC, $\chi 2$ test was used. One-way ANOVA was used for comparisons of number of defibrillation, duration of survival, and LV infarct size. For comparisons of all other time-based variables, repeated measures ANOVA with Holm-Sidak's multiple comparison was used.