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TRANSLATIONAL RESEARCH

Delayed Argon Administration Provides Robust Protection Against Cardiac Arrest-Induced Neurological Damage

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Abstract
Introduction Argon at a dosage of 70 % is neuroprotective, when given 1 h after cardiac arrest (CA) in rats. We investigated if a neuroprotective effect of argon would also be observed, when administration was delayed.
Methods Twenty-four male Sprague–Dawley rats, weighing between 400 and 500 g were subjected to 7 min of CA and 3 min of cardiopulmonary resuscitation. Animals were randomized to receive either 1 h of 70 % argon ventilation 1 h ($n = 8$) or 3 h ($n = 8$) after return of spontaneous circulation or no argon treatment ($n = 8$). For all animals, a neurological deficit score (NDS) was calculated daily for 7 days following the experiment. On day 8, rats were re-anesthetized and transcardially perfused before brains were harvested for histopathological analyses.
Results All animals survived. Control animals exhibited severe neurologic dysfunction at all time points as measured with the NDS. Argon-treated animals showed significant improvements in the NDS through all postoperative days, even when argon administration was delayed for 3 h. This was paralleled by a significant reduction in the neuronal damage index in the neocortex and the hippocampal CA 3/4 region in argon-treated animals, regardless of the timing of argon administration. However, animals of the delayed argon administration group additionally showed significant reductions in the basal ganglia in comparison with control animals.
Conclusion Our study demonstrates that a 1-h application of argon provided a significant reduction in histopathological damage, associated with a marked improvement in functional neurologic recovery even when treatment was delayed for 3 h. This is highly significant with regard to clinical situations, where argon treatment cannot be provided timely.

Keywords Cardiopulmonary resuscitation · Hypoxia–ischemia, Brain · Reperfusion injury · Noble gases · Argon · Neuroprotective agents

Introduction

Noble gas-mediated neuroprotection has gained considerable attention in the last decade and especially xenon has been extensively studied. Numerous preclinical studies have repeatedly demonstrated both functional and structural improvements in various animal models of neurological injury including stroke, hypoxic-ischemic encephalopathy, and cardiac arrest (CA) [1–7]. Given the negligible side effects of the gas, which are well known from its use as an anesthetic [8], these remarkable results have translated in phase II clinical trials exploring the effects of xenon as an adjunct to mild therapeutic hypothermia in neonates suffering from hypoxic-ischemic encephalopathy and in adults resuscitated from CA. First results revealed the feasibility and cardiac safety of xenon treatment combined with mild therapeutic hypothermia in survivors of out-of-hospital cardiac arrest [9]. However,

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data on neurological outcome are not yet available. Pending the results of these investigations, it has to be noted that xenon's delivery is cumbersome in the intensive care unit because specialized ventilators are needed to economically administer the gas. This is due to the rarity of the gas, which makes it costly and might preclude a widespread clinical use.

In contrast, argon is much more abundant in the atmosphere and, therefore, available at a significantly lower price. Interestingly, accumulating data from preclinical studies provide evidence that argon albeit the lack of an anesthetic effect has also organ-protective properties [10–13]. Our own group has previously shown that rats exposed to a single 1 h administration of 70 % argon demonstrated significant and persistent reductions in CA-induced neurological dysfunction which was accompanied by a concomitant decrease in the numbers of damaged neurons in hippocampal and cortical regions of the brain [14]. However, the time window used in this study was relatively short (i.e., 1 h after successful resuscitation) and might, therefore, not be applicable in situations, where treatment cannot be provided timely. The present study was therefore designed to address this issue in a rodent model of CA-induced neurological damage. We hypothesized that the beneficial effects of argon would also be observed after a prolonged period (i.e., 3 h post-arrest).

Materials and Methods

Experiments were performed in 24 male Sprague–Dawley rats (Charles River, Germany) weighing between 400 and 500 g. Animals were housed in adequately spaced cages (60 cm × 40 cm; type 2000; Tecniplast; Buguggiate; Italy) with a 12 h light–dark cycle from 6 am to 6 pm. Animals had free access to water and food prior to the study. The study protocol was approved by the appropriate institution (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen; Recklinghausen; Germany), and the experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals formulated by the National Research Council (National Academies Press, 1996). In addition, all reported data and outcomes are in accordance with the "Utstein style guidelines for uniform reporting of laboratory CPR research" [15].

Animal Preparation

On the experimental day, rats were anesthetized with an intraperitoneal injection of pentobarbital (45 mg kg^{-1}). Additional doses (10 mg kg^{-1}) of pentobarbital were administered if signs of animal discomfort were noted; i.e., sudden rise in heart rate or respiratory rate or movements of the tail or paws. The animal's chest and back were thoroughly shaved to allow for direct contact of the paddles used for defibrillation during CPR.

After placing on a surgical board in the supine position, the trachea was orally intubated using a modified 14 G cannula (Abbocath-T, Abbott Hospital Division, North Chicago, IL, USA) as previously described [16]. Animals were mechanically ventilated (Servo Ventilator 900 C, Siemens ELEMA, München, Deutschland) with an FiO_2 of 0.21. Respiratory frequency was adjusted to maintain end-tidal PCO_2 between 35 and 40 mmHg, which was continuously monitored using an infrared CO_2 analyzer (Cap Star 100, CWE Inc., Ardmore, PA, USA). A three lead electrocardiogram was continuously measured by monopolar needle electrodes (MLA1204 Needle Electrodes, ADinstuments, Oxford, UK). The right jugular vein and right femoral artery were surgically exposed and cannulated with polyethylene catheters (PE 50) and connected to high-sensitivity transducers (Capto SP 844 Physiologic Pressure Transducer, Capto Inc., Skoppum, Norway) for the measurement of right atrial and mean arterial pressures (MAP), respectively. A thermocouple microprobe (IT-18, Physitemp Instruments, Clifton, NJ, USA) was placed into the abdominal aorta via the left femoral artery. Cardiac output (CO) was measured with the transpulmonary thermodilution technique using this microprobe. Blood temperature was monitored and maintained between 37 and 37.5 °C with the aid of a heating lamp. The left femoral vein was also cannulated with an additional PE 50 catheter to allow for administration of fluids and epinephrine during CPR. All catheters were flushed intermittently with saline solution containing 2 IU ml^{-1} of heparin.

Experimental Procedure

Ventricular fibrillation (VF) was induced by transesophageal electrical stimulation. After placing the electrode using fluoroscopy, alternating current (10 V, 50 Hz) was delivered to the heart using a commercially available fibrillator (Fi 20 M, Stockert GmbH, Freiburg, Germany). CA was confirmed by an abrupt decrease in MAP to less than 20 mmHg. Simultaneously, ventilation was stopped. After 7 min of untreated CA, CPR was initiated including mechanical ventilation with an FiO_2 of 1.0 at a respiratory rate of 50 min^{-1} and chest compressions delivered by a custom made mechanical thumper at a stroke rate of 200 min^{-1}. An intravenous bolus of 0.02 mg kg^{-1} epinephrine was administered via the femoral access 30 s after starting chest compressions. After 3 min of CPR, external defibrillation with 5 J (Zoll MSeries, Zoll Medical Corporation, Chelmsford, MA, USA) was attempted up to three times. If restoration of spontaneous circulation

(ROSC) was not achieved chest compressions for 1 min and administration of epinephrine at the same dosage were repeated before another series of direct current counter shocks (again upto three times) was delivered. ROSC was confirmed by spontaneous cardiac rhythm in conjunction with a rise in mean arterial pressure to greater than 50 mmHg. 1 h after successful resuscitation, FiO_2 was reduced to 0.3 and the animals were randomly assigned into groups. Only animals achieving ROSC were included in the study. Animals of the argon groups received either 1 h of 70 % argon in 30 % oxygen 1 h ($n = 8$) or 3 h ($n = 8$) after ROSC. Argon gas was administered using prespecified gas cylinders containing the desired concentration (Linde Gas Therapeutics, Unterschleißheim, Germany). Animals of the control group ($n = 8$) did not receive any argon treatment and were ventilated with 30 % oxygen in 70 % nitrogen. Randomization was performed using the sealed envelope method. Overall, animals were ventilated for 5 h following ROSC. At the end of the experiment, all animals received a single subcutaneous injection of 0.1 mg kg^{-1} buprenorphine for pain relief and were weaned from the ventilator. Following extubation, animals were observed for approximately 30 min to ensure adequate spontaneous breathing before being returned to their cages.

Measurements

Ischemia time was calculated as the sum of the duration of VF, CPR, and the time needed to achieve ROSC. Heart rate, MAP, end-tidal CO_2, and blood temperature were continuously recorded on a multichannel recorder (Power Lab, AD Instruments, Spechbach, Germany). CO was calculated by bolus injections of 200 µL of cold saline (4 °C) into the right atrium. Two consecutive measurements were performed and the results averaged (Cardiac Output Pod, AD Instruments, Spechbach, Germany).

Arterial blood samples were drawn at baseline, 30 min and 4 h after ROSC. Arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) partial pressures as well as glucose and lactate levels were measured using a conventional blood gas analyzer (ABL700, Radiometer Copenhagen, Denmark).

Neurological Testing

Neurological Deficit Score

On the 7 days following CPR, neurological performance was evaluated daily using a neurological deficit score (NDS) previously established in an asphyxial CA model in rats [17]. The test consists of six items representing the level of consciousness, respiration, cranial nerves, motor and sensory function, and coordination. Each item is graded depending on the severity and given a score. The score ranges from 0 (worst neurological impairment) to 500 (no neurological impairment). Blinding of the independent investigator was achieved by assigning numbers to the animals.

Open Field Test

The open field test is commonly used in rodents as a qualitative and quantitative measure of stress-induced anxiety using the willingness to explore a previously unknown environment depending on general locomotor activity [18]. In brief, 4 days after CA animals were placed in a brightly illuminated custom made box consisting of a rectangular arena (50 cm × 50 cm) divided in 16 zones of identical size and opaque 35 cm high walls. The test was always started at the same daytime (i.e., 12 am) by placing the rat in the center of the arena. Using a computerized tracking system (Any Maze video Tracking System Version 4.72, Stoelting Coorporation, Illinois, USA), the animal's reactivity was recorded for 5 min by a video camera mounted above the field. The time the animals were mobile, moved along the walls, or rested in the middle and corners was recorded.

Neurohistopathology

8 days after successful resuscitation, rats were re-anesthetized as described above. A midline thoracotomy was performed, and the animals were transcardially perfused with 100 ml of NaCl 0.9 %. Brains were then carefully removed and transsagitally cut in half. Right hemispheres were postfixed in buffered 4 % paraformaldehyde. Standardized coronal slices were taken at a thickness of 2 mm resulting in a total of eight slices per brain. The anterior and posterior CA 3/4 sectors of the hippocampus, basal ganglia, and neocortex were chosen as regions of interest and analyzed by an experienced neuropathologist blinded to the animals treatment assignment. Conventional hematoxylin/eosin (HE) and NeuN (Mouse anti-Neuronal Nuclei, monoclonal, Company: Millipore, Cat # MAB 377) staining was performed. A Neuronal damage index was semiquantitatively assessed by determining the proportion of neuronal cells showing shrunken and/or hypereosinophilic cytoplasm (HE staining) in combination with a loss of NeuN-immunoreactivity and summarized in a score as previously established [14]: 0–5 % = 1, 5–10 % = 2, 10–20 % = 3, 20–30 % = 4, 30–40 % = 5, 40–50 % = 6, 50–60 % = 7, 60–70 % = 8, 70–80 % = 9, 80–90 % = 10, 90–100 % = 11.

Statistical Analysis

Normal distribution of the data, accept the NDS, was confirmed using the Kolmogorov–Smirnov test. Group comparisons at the given time points were performed using a one-way analysis of variance, followed by post hoc bonferroni testing. Data of the NDS were not normally distributed as tested with the Kolmogorov–Smirnov test. In this case, group comparisons at the given time points were performed using a non-parametric Kruskal–Wallis Test, followed by pairwise post hoc testing. In all cases, a $p \leq 0.05$ was considered to indicate statistical significance.

Results

No significant differences were observed with regard to hemodynamics, variables of gas exchange or glucose concentrations between argon-treated animals and the control group at baseline (Table 1). Ischemia time was comparable in all groups (Argon 1 h post ROSC: 644 ± 35 s vs. Argon 3 h post ROSC: 621 ± 31 s vs. Control: 644 ± 37 s). In all animals, successful CPR resulted in a dramatic decrease in CO 30 min after CA, which was paralleled by a significant decrease in MAP and heart rate in comparison to baseline parameters. Tissue ischemia as indicated by a marked increase in lactate levels 30 min post-resuscitation returned to near baseline levels 4 h post-arrest. Blood temperature was in all animals tightly around 37.2 °C during the whole observation period.

We observed severe neurological dysfunction as measured with the NDS in all control animals during the 7 days after CA and CPR. Strikingly, argon-treated animals showed a significantly better NDS through all postoperative days, even when argon administration was delayed for 3 h (Fig. 1 and Fig. 1S).

This was paralleled by more mobile episodes in the open field test documented for all argon-treated animals, however, only being significant for animals receiving argon delayed (Fig. 2). In contrast and independent of the timing, argon-treated animals moved significantly more frequently along the walls, while control animals stayed in the corner of the open field (Fig. 2).

Neurohistopathological evaluation revealed significant reductions in the neuronal damage index in the neocortex (Fig. 4a) and the hippocampal CA 3/4 sector (Fig. 4b) in argon-treated animals regardless of the timing of argon administration (Fig. 3). Animals of the delayed argon administration group additionally showed significant reductions in the basal ganglia in comparison to control animals as displayed in Figs. 3 and 4c.

Table 1 Physiologic data of control and Argon-treated animals to baseline and after cardiopulmonary resuscitation

	BL	PR 30 min	PR 4 h
HR (bpm)			
Control	452 ± 22	350 ± 40	391 ± 52
Argon 1 h post ROSC	436 ± 25	371 ± 63	430 ± 25
Argon 3 h post ROSC	449 ± 39	420 ± 15	428 ± 49
MAP (mmHg)			
Control	147 ± 5	95 ± 1	107 ± 12
Argon 1 h post ROSC	143 ± 8	93 ± 13	112 ± 15
Argon 3 h post ROSC	144 ± 7	96 ± 14	108 ± 13
CO (ml/min)			
Control	252 ± 14	129 ± 26	174 ± 27
Argon 1 h post ROSC	251 ± 14	141 ± 35	191 ± 54
Argon 3 h post ROSC	251 ± 30	148 ± 28	186 ± 29
Hgb (g/dl)			
Control	14.5 ± 0.6	15.3 ± 0.4	13.3 ± 1.0
Argon 1 h post ROSC	14.1 ± 1.1	15.1 ± 1.5	14.7 ± 4.3
Argon 3 h post ROSC	15.5 ± 0.5	15.4 ± 1.4	14.3 ± 1.1
Glucose (mmol/L)			
Control	7.8 ± 0.9	12.1 ± 3.8	9.5 ± 0.9
Argon 1 h post ROSC	8.0 ± 0.5	9.0 ± 1.8	8.6 ± 1.4
Argon 3 h post ROSC	9.1 ± 0.5	14.1 ± 4.4	10.8 ± 1.4
Lactate (mmol/L)			
Control	0.8 ± 0.2	2.5 ± 1.2	0.9 ± 0.3
Argon 1 h post ROSC	0.9 ± 0.2	2.6 ± 0.7	0.9 ± 0.3
Argon 3 h post ROSC	1.3* ± 0.4	3.1 ± 1.6	1.0 ± 0.3
PaO_2 (mmHg)			
Control	104 ± 15	425 ± 71	138 ± 12
Argon 1 h post ROSC	126 ± 40	348 ± 100	163 ± 17
Argon 3 h post ROSC	125 ± 17	409 ± 82	172* ± 35
$PaCO_2$ (mmHg)			
Control	37 ± 3	44 ± 2	36 ± 4
Argon 1 h post ROSC	40 ± 5	41 ± 5	38 ± 5
Argon 3 h post ROSC	42 ± 4	43 ± 3	39 ± 2
Temperature (°C)			
Control	37.2 ± 0.1	37.2 ± 0.2	37.2 ± 0.5
Argon 1 h post ROSC	37.2 ± 0.2	37.2 ± 0.2	37.2 ± 0.9
Argon 3 h post ROSC	37.2 ± 0.2	37.2 ± 0.1	37.1 ± 0.1

HR heart rate, *MAP* mean arterial pressure, *CO* cardiac output, *PR* time post-resuscitation

* $p < 0.05$ vs. Control

Discussion

Our results are in accordance to our previous findings and demonstrate that argon has still neuroprotective effects beyond 3 h following successful CPR. All argon-treated animals showed significantly better neurological outcome than control animals. This was paralleled by significant reductions in the neuronal damage index in the neocortex

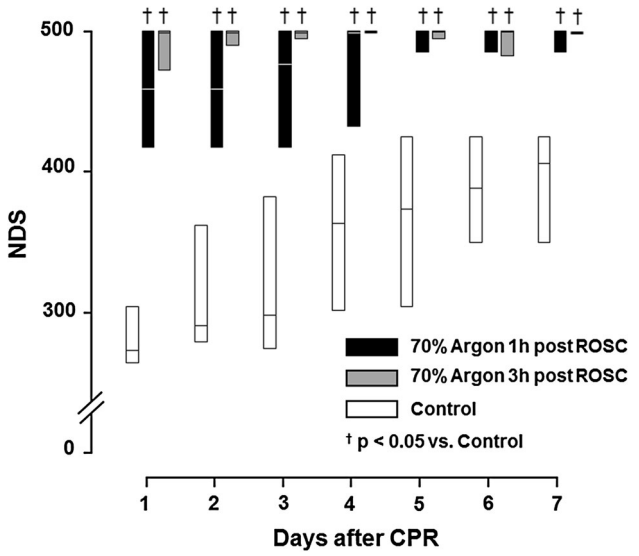


Fig. 1 Neurological deficit score (NDS) on all days after cardiopulmonary resuscitation (CPR). Severe neurological dysfunction could be measured with the NDS in all control animals ($n = 8$). Argon-treated animals ($n = 8$) showed a significantly better NDS through all postoperative days, even when argon administration was delayed for 3 h. $^{\dagger}p < 0.05$ vs control; *white/black horizontal lines* represent the median; boxes represent the 25–75 % interquartile range

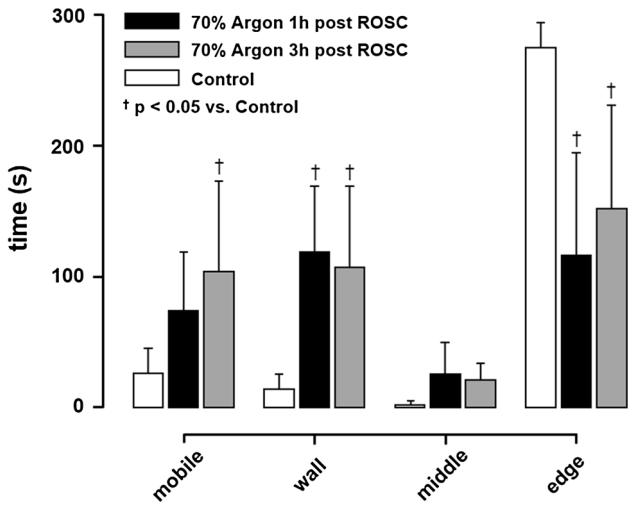


Fig. 2 The open field test on day 4 after cardiopulmonary resuscitation (CPR) evaluates the animals normal exploratory behavior and spontaneous locomotor activity. More mobile episodes were documented for all argon-treated animals; however, only being significant for those receiving argon delayed ($n = 8$). All argon-treated animals ($n = 16$) moved significantly more time along the walls than control animals ($n = 8$), which stayed more in the corners. $^{\dagger}p < 0.05$ vs control; mean ± SD

and the hippocampal CA 3/4 sector, regardless of the timing of argon administration. We believe this to be highly significant with regard to clinical situations, where argon treatment might be delayed.

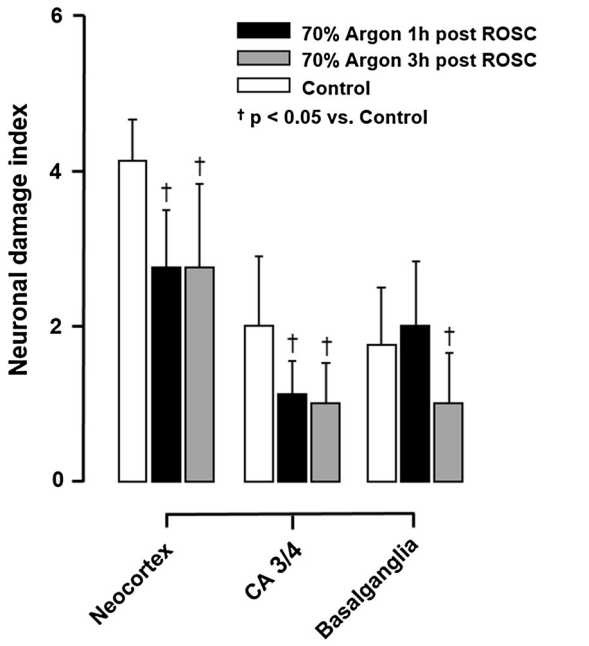


Fig. 3 Histopathological evaluation of the regions of interest. Argon-treated animals showed significant reductions in the neuronal damage index in the neocortex and the hippocampal CA 3/4 sector, independent of the timing of administration (Argon 1 h post ROSC $n = 8$; Argon 3 h post ROSC $n = 8$). Only animals that received argon delayed ($n = 8$) additionally showed significant reductions in the basal ganglia in comparison to control ($n = 8$) animals $^{\dagger}p < 0.05$ vs control: mean ± SD

In the last years, several in vitro and cumulative in vivo studies suggested argon's potency to protect renal and nervous tissues against hypoxic-ischemic insults [10–14, 19].

In a rodent middle cerebral artery occlusion (MCAO) model of 2 h duration Ryang and co-workers found that an intra-ischemic (i.e., 1 h after induction of MCAO) argon administration of 1 h provides both cortical and subcortical neuroprotection [13]. Our results support these findings and show that even when post- arrest argon administration is delayed, neuronal damage is still significantly reduced in the neocortex and the hippocampal CA 3/4 sector. This is in accordance with David and colleagues, who recently demonstrated, that argon, when given 3 h after reperfusion of a 60-min MCAO, reduced cortical brain damage. However, they also report an increase in subcortical brain damage. Strikingly, this was accompanied by a lack of improvement in clinical neurological deficits [20]. This differs to our observation, in which we found a marked and significant improvement in neurological performance. This effect is probably not explained by a different timing in argon delivery, as we still observed improved neurological and neurohistopathological outcome, even when argon administration was delayed for 3 h. However, it might be attributable to the different experimental models (i.e., focal

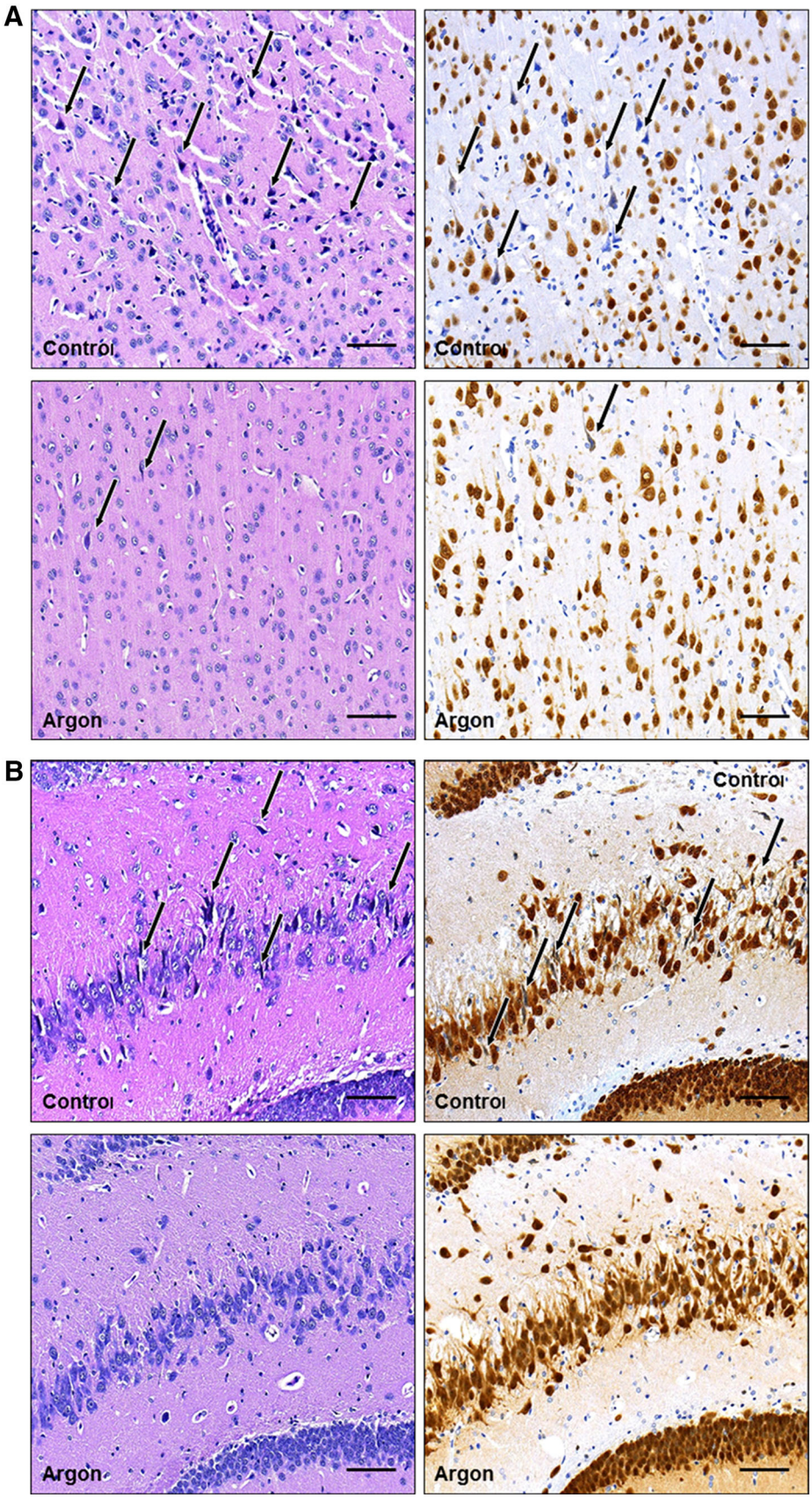
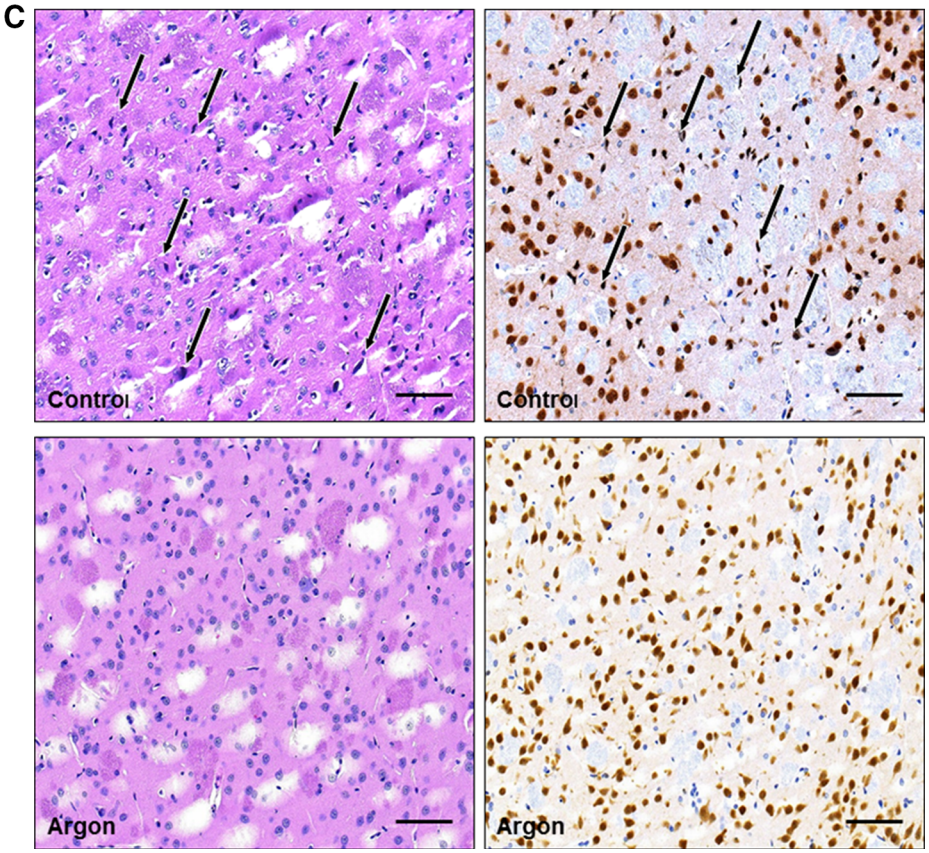


Fig. 4 Representative photomicrographs in HE and NeuN staining of the neocortex (**a**), the hippocampus sector CA 3/4 (**b**) and the basal ganglia (**c**) showing numerous ischemically damaged neurons (*black arrows*) in a control animal and almost no necrosis in an argon-treated animal. *Scale bars* 90 μm

Fig. 4 continued



vs global cerebral ischemia) and differences in total ischemia time (i.e., 7 vs 60 min).

Loetscher and co-workers provide further evidence that argon is effective after a delay. They demonstrated in in vitro models involving either a focal mechanical trauma or oxygen glucose deprivation (OGD) that argon significantly reduced neuronal damage even when applied 2 or 3 h after experimental injury [11]. Argon's neuroprotective properties in the setting of OGD have also been described by David et al. [20], who additionally demonstrated that neuroprotection by argon increased as a function of time during the 3 h of "reperfusion." Furthermore, they provide evidence that argon also blocks NMDA-induced excitotoxicity in a concentration-dependent manner.

Interestingly, delayed argon administration seems to be even more effective than early treatment. Animals treated delayed tended to show a better functional outcome than early-treated animals in the NDS (Fig. 1) and the open field test (Fig. 2). In the NDI, only animals that received argon delayed showed a significant protection of the basal ganglia (Fig. 3). David et al. [21] showed that argon at a concentration of 75 % increases tissue plasminogen activator activity, favoring thrombolysis, while concurrently increasing proteolysis and brain damage. If argon is given late (i.e., 3 h after reperfusion), the critical endogenous peak of tissue plasminogen activation that corresponds to early reperfusion might have resolved and would explain the observed benefits. However, although there is increasing evidence that argon mediates neuroprotection, the mechanisms involved are less well described. Recently, Harris and colleagues investigated the importance of the two pore domain potassium channels in the setting of traumatic brain injury and revealed that argon lacks any effect on TREK-1 currents [22]. A recently published study of our own group demonstrated that another subtype of potassium channels—namely ATP dependent potassium channels—does not contribute to argon-mediated neuroprotection either [23]. In addition, argon's neuroprotective properties are not reversed by the amino-acid glycine [22]. One might presume that this makes it unlikely that argon acts as antagonist at the NMDA receptor, which is considered the major site for xenon-mediated neuroprotection. However, on the subcellular level, recent investigations proposed involvement of the ERK pathway [24], an essential component of NMDA receptor signal transduction controlling neuroplasticity [25]. The only suggested mechanisms for argon's neuroprotective potency from pharmacological and physiological studies are its oxygen-like effect [26, 27] and its agonistic action on the gamma-aminobutyric acid receptor, acting particularly but not only at the benzodiazepine site of this receptor [28].

We recognize several limitations in our study. The design of the present study does not allow drawing any conclusions, if longer administration periods would provide a greater degree of protection. Furthermore, post-resuscitation intensive care treatment, which is usually required in human settings, has not been provided and might have influenced results. In addition, we did not verify end-expiratory or blood concentrations of argon. However, all argon-treated animals were ventilated identically with predefined and calibrated argon mixtures containing 70 % of the gas. Furthermore, functional outcome has only been followed up until 7 days after CA. It may well have been that control animals would improve over a longer period of time, which could be thought to limit the value of argon treatment. However, improvements in functional outcome over time in control animals could be also attributable to the high plasticity of rat brains as opposed to the possibility of a lack in argon's neuroprotection. Indeed, in humans early neurological failure is the most common mode of death in CA victims [29].

In addition, we have yet not studied the neuroprotective effects of argon in comparison with xenon, which can be considered the gold standard in noble gas-mediated neuroprotection. However, the finding that argon resulted in a robust functional improvement is important, since the widespread use of xenon may be limited regarding its costs. In addition, our model might represent a milder form of postanoxic injury and we cannot conclude if argon might also exert beneficial effects ischemic injury exceeding 10 min of CA. Finally, translation of argońs efficacy to protect nervous tissues of higher vertebrates including humans is lacking, although Ristagno et al. [30] recently provided evidence that argon has also neuroprotective effects in pigs subjected to CA.

Conclusions

In summary, our study demonstrates that a 1-h application of argon provided a significant reduction in histopathological damage, associated with a marked improvement in functional neurologic recovery even when treatment was delayed for 3 h. This is highly significant with regard to clinical situations, where argon treatment cannot be provided timely.

Conflict of interest Anne Brücken, Pinar Kurnaz, Christian Bleilevens, Matthias Derwall, Joachim Weis, Kay Nolte, Rolf Rossaint, and Michael Fries declare that they have no conflict of interest.

References

1. Coburn M, Maze M, Franks NP. The neuroprotective effects of xenon and helium in an in vitro model of traumatic brain injury. Crit Care Med. 2008;36:588–95.
2. Dingley J, Tooley J, Porter H, Thoresen M. Xenon provides short-term neuroprotection in neonatal rats when administered after hypoxia-ischemia. Stroke. 2006;37:501–6.
3. Luo Y, Ma D, Ieong E, et al. Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. Anesthesiology. 2008;109:782–9.
4. Ma D, Wilhelm S, Maze M, Franks NP. Neuroprotective and neurotoxic properties of the 'inert' gas, xenon. Br J Anaesth. 2002;89:739–46.
5. Schmidt M, Marx T, Gloggl E, Reinelt H, Schirmer U. Xenon attenuates cerebral damage after ischemia in pigs. Anesthesiology. 2005;102:929–36.
6. Fries M, Nolte KW, Coburn M, et al. Xenon reduces neurohistopathological damage and improves the early neurological deficit after cardiac arrest in pigs. Crit Care Med. 2008;36:2420–6.
7. Fries M, Brücken A, Cizen A, et al. Combining xenon and mild therapeutic hypothermia preserves neurological function after prolonged cardiac arrest in pigs. Crit Care Med. 2012;40:1297–303.
8. Derwall M, Coburn M, Rex S, Hein M, Rossaint R, Fries M. Xenon: recent developments and future perspectives. Minerva Anestesiol. 2009;75:37–45.
9. Arola OJ, Laitio RM, Roine RO, et al. Feasibility and cardiac safety of inhaled xenon in combination with therapeutic hypothermia following out-of-hospital cardiac arrest. Crit Care Med. 2013;41:2116–24.
10. Yarin YM, Amarjargal N, Fuchs J, et al. Argon protects hypoxia-, cisplatin- and gentamycin-exposed hair cells in the newborn rat's organ of Corti. Hear Res. 2005;201:1–9.
11. Loetscher PD, Rossaint J, Rossaint R, et al. Argon: neuroprotection in in vitro models of cerebral ischemia and traumatic brain injury. Crit Care. 2009;13:R206.
12. Jawad N, Rizvi M, Gu J, et al. Neuroprotection (and lack of neuroprotection) afforded by a series of noble gases in an in vitro model of neuronal injury. Neurosci Lett. 2009;460:232–6.
13. Ryang YM, Fahlenkamp AV, Rossaint R, et al. Neuroprotective effects of argon in an in vivo model of transient middle cerebral artery occlusion in rats. Crit Care Med. 2011;39:1448–53.
14. Brücken A, Cizen A, Fera C, et al. Argon reduces neurohistopathological damage and preserves functional recovery after cardiac arrest in rats. Br J Anaesth. 2013;110(Suppl 1):i106–12.
15. Idris AH, Becker LB, Ornato JP, et al. Utstein-style guidelines for uniform reporting of laboratory CPR research. A statement for healthcare professionals from a Task Force of the American Heart Association, the American College of Emergency Physicians, the American College of Cardiology, the European Resuscitation Council, the Heart and Stroke Foundation of Canada, the Institute of Critical Care Medicine, the Safar Center for Resuscitation Research, and the Society for Academic Emergency Medicine. Resuscitation. 1996;33:69–84.
16. Stark RA, Nahrwold ML, Cohen PJ. Blind oral tracheal intubation of rats. J Appl Physiol. 1981;51:1355–6.
17. Neumar RW, Bircher NG, Sim KM, et al. Epinephrine and sodium bicarbonate during CPR following asphyxial cardiac arrest in rats. Resuscitation. 1995;29:249–63.
18. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 2003;463:3–33.
19. Zhuang L, Yang T, Zhao H, Fidalgo A, Vizcaychipi MP, Sanders R, Yu B, Takata M, Johnson M, Ma D. The protective profile of argon, helium, and xenon in a model of neonatal asphyxia in rats. Crit Care Med. 2012;40:1724–30.
20. David HN, Haelewyn B, Degoulet M, Colomb DG Jr, Risso JJ, Abraini JH. Ex vivo and in vivo neuroprotection induced by

argon when given after an excitotoxic or ischemic insult. PLoS One. 2012;7:e30934.
21. David HN, Haelewyn B, Risso JJ, Abraini JH. Modulation by the noble gas argon of the catalytic and thrombolytic efficiency of tissue plasminogen activator. Naunyn Schmiedebergs Arch Pharmacol. 2013;386:91–5.
22. Harris K, Armstrong SP, Campos-Pires R, Kiru L, Franks NP, Dickinson R. Neuroprotection against traumatic brain injury by xenon, but not argon, is mediated by inhibition at the *n*-methyl-D-aspartate receptor glycine site. Anesthesiology. 2013;119(5):1137–48.
23. Brücken A, Kurnaz P, Bleilevens C, et al. Dose dependent neuroprotection of the noble gas argon after cardiac arrest in rats is not mediated by K-Channel opening. Resuscitation. 2014;85(6):826–32.
24. Fahlenkamp AV, Rossaint R, Haase H, et al. The noble gas argon modifies extracellular signal-regulated kinase 1/2 signaling in neurons and glial cells. Eur J Pharmacol. 2012;674:104–11.
25. Krapivinsky G, Krapivinsky L, Manasian Y, et al. The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1. Neuron. 2003;40:775–84.
26. Soldatov PE, D'Iachenko AI, Pavlov BN, Fedotov AP, Chuguev AP. Survival of laboratory animals in argon-containing hypoxic gaseous environments. Aviakosm Ekolog Med. 1998;32:33–7.
27. Antonov AA, Ershova TA. Retention of the skill to perform adaptive bio-control of bioelectrical activity synchronization in the human brain cortex in an argon-nitrogen-oxygen atmosphere with various oxygen content. Aviakosm Ekolog Med. 2009;43:27–31.
28. Abraini JH, Kriem B, Balon N, Rostain JC, Risso JJ. Gamma-aminobutyric acid neuropharmacological investigations on narcosis produced by nitrogen, argon, or nitrous oxide. Anesth Analg. 2003;96:746–9.
29. Laver S, Farrow C, Turner D, Nolan J. Mode of death after admission to an intensive care unit following cardiac arrest. Intensive Care Med. 2004;30:2126–8.
30. Ristagno G, Fumagalli F, Russo I, et al. Postresuscitation treatment with argon improves early neurological recovery in a porcine model of cardiac arrest. Shock. 2014;41:72–8.