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NEUROPROTECTION

Argon reduces neurohistopathological damage and preserves functional recovery after cardiac arrest in rats

A. Brücken[1]*, A. Cizen[2], C. Fera[2], A. Meinhardt[3], J. Weis[3], K. Nolte[3], R. Rossaint[2], T. Pufe[4], G. Marx[1] and M. Fries[2]

[1] Department of Surgical Intensive Care, [2] Department of Anaesthesiology, [3] Institute for Neurohistopathology, and [4] Department of Anatomy and Cell Biology, University Hospital RWTH Aachen, Aachen, Germany

* Corresponding author. E-mail: abruecken@ukaachen.de

Editor's key points

- Xenon protects against neurological injury but is expensive.
- Argon is more available and cheaper.
- Inhalation of 70% argon was used in rats after cardiac arrest.
- Rats treated with argon had better neurological outcomes than control rats.

Background. Xenon has profound neuroprotective effects after neurological injury and is currently undergoing phase 2 clinical trials in cardiac arrest patients. However, xenon is very costly, which might preclude its widespread use. We hypothesized argon, which is more available, might also protect central nervous tissues and allow better functional recovery in a rodent model of global cerebral ischaemia.

Methods. Fourteen male Sprague–Dawley rats were subjected to 7 min of cardiac arrest and 3 min of cardiopulmonary resuscitation (CPR). One hour after successful CPR, animals were randomized to either ventilation with 70% argon in oxygen ($n = 7$) for 1 h or 70% nitrogen (controls, $n=7$). A neurological deficit score (NDS) was calculated daily for the following 7 days, then the animals were killed and the brains harvested for histopathological analyses.

Results. All animals survived. Control rats had severe neurological dysfunction, while argon-treated animals showed significant improvements in the NDS at all time points. This was paralleled by a significant reduction in the neuronal damage index in the neocortex and the hippocampal CA 3/4 region.

Conclusions. Our study demonstrates that a single 1 h application of 70% argon significantly reduced histopathological damage of the neocortex and hippocampus, associated with a marked improvement in functional neurological recovery.

Keywords: argon; cardiopulmonary resuscitation; hypoxia–ischaemia, brain

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Approximately 1.5 million people undergo cardiac arrest (CA) every year.[1 2] Although initial successful cardiopulmonary resuscitation (CPR) is increasing, still 70% of patients subsequently die in hospital despite intensive care treatment.[3 4] The high mortality rate is attributable to the severity of post-cardiac arrest syndrome which includes coagulation abnormalities, inflammation, and endothelial activation.[5] The resulting degree of neurological deficit ultimately represents the most important factor for overall survival in cardiac arrest patients.[6] Mild therapeutic hypothermia (MTH) is the only treatment to reduce neurological sequelae and mortality after CA.[7 8]

The noble gas xenon, although considered inert, has biological activities, including potent anaesthetic effects.[9] Over the last decade, there have been numerous reports on the organ-protecting properties of xenon in different experimental models of neurological injury, including stroke, traumatic brain injury, cardiac arrest, and hypoxic-ischaemic encephalopathy.[10–14] We have also recently demonstrated neuroprotective effects of xenon alone and in combination with mild therapeutic hyothermia in a porcine model of cardiac arrest.[15 16] The clinical use of xenon either as an anaesthetic or as an organprotective agent is limited by its cost. Argon may represent a promising alternative to xenon, given its high atmospheric concentration, which makes it inexpensive and widely available. Only few *in vitro* studies have examined the ability of argon to protect nervous tissues,[17–19] and data regarding its *in vivo* effects are even more limited.[20 21]

The aim of the present study was to evaluate the effects of argon on neurological outcome in a rodent model of cardiac arrest. We hypothesized that argon would preserve functional recovery which would be paralleled by decreased neurohistopathological injury compared with untreated control animals.

Methods

Experiments were performed in 14 male Sprague–Dawley rats (Charles River, Germany) weighing between 400 and 500 g. Animals had free excess to water and food before

the study and were housed with a 12 h light–dark cycle. The study protocol had institutional approval (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) and are reported according to the ARRIVE guidelines (National Centre for the Replacement, Refinement and Reduction of Animals in Research, 2010).

Animal preparation

On the experimental day, rats were anaesthetized with an intraperitoneal injection of pentobarbital (45 mg kg^{-1}). Additional doses (10 mg kg^{-1}) were administered if signs of stress occurred such as increases in heart rate or respiratory frequency or spontaneous movements. The chest and back were thoroughly shaved to allow for direct contact of the paddles used for defibrillation during CPR as soon as the rats lost consciousness.

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.[22] Animals were mechanically ventilated (Servo Ventilator 900 C, Siemens ELEMA, München, Deutschland) with an F_{IO_2} of 0.21. Respiratory frequency was adjusted to maintain end-tidal P_{CO_2} between 35 and 40 mm Hg, which was continuously monitored using an infrared CO_2 analyzer (Cap Star 100, CWE Inc., Ardmore, PA, USA). A three-lead ECG was measured continuously by monopolar needle electrodes (MLA1204 Needle Electrodes, ADInstruments, Oxford, UK). The right jugular vein and the 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 was also measured with the transpulmonary thermodilution technique using this microprobe. Blood temperature was monitored and temperature maintained between 37°C 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 transoesophageal 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). Cardiac arrest was confirmed by an abrupt decrease in MAP below 20 mm Hg. Simultaneously, ventilation was stopped. After 7 min, CPR was initiated, including restoration of ventilation with an F_{IO_2} of 1.0 at a respiratory rate of 50 bpm, and chest compression was delivered by a custom-made mechanical thumper at a stroke rate of 200 min^{-1}. A bolus of 0.02 mg kg^{-1} epinephrine was administered i.v. 30 s after starting chest compressions. After 3 min of CPR, external defibrillation (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 and administration of epinephrine at the same dosage were repeated for 1 min before another series of direct current counter shocks was delivered. ROSC was confirmed by spontaneous cardiac rhythm in conjunction with an increase in MAP>50 mm Hg. One hour after successful resuscitation, F_{IO_2} was reduced to 0.3 and the animals were randomly assigned to receive either 1 h ventilation with 70% argon or 1 h of ventilation with 70% nitrogen (control group). Randomization was performed using the sealed envelope method. After the end of the treatment period, animals in both groups received 70% nitrogen in 30% oxygen for an additional 3 h. At the end of the experiment, all animals received a single subcutaneous injection of 0.1 mg kg^{-1} buprenorphin for pain relief and were weaned from the ventilator. After extubation, animals were observed for ~30 min to ensure adequate spontaneous breathing before being returned to their cages.

Measurements

Ischaemia 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, ADInstruments, Spechbach, Germany). Cardiac output was calculated after 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, ADInstruments, Spechbach, Germany).

Arterial blood samples were drawn at baseline, 30 min, and 4 h after ROSC. Arterial oxygen (Pa_{O_2}) and carbon dioxide (Pa_{CO_2}) partial pressures and also glucose and lactate levels were measured using a conventional blood gas analyzer (ABL700, Radiometer Copenhagen, Denmark). The remaining blood (0.5 ml) was centrifuged and the serum was immediately stored at −80°C for subsequent measurement of brain-derived neurotrophic factor (BDNF), adrenocorticotropic hormone (ACTH), and prolactin using xMAP multiplex technology (Luminex 100/200 System, Austin, TX, USA).[23] An additional serum sample was drawn on day 8 when animals were re-anaesthesized for preparation of the brain.

Neurological testing

Neurological deficit score

On each of the 7 days after CPR, neurological performance was evaluated using a neurological deficit score (NDS) previously established in rats in an asphyxial cardiac arrest model.[24] The test comprises six measures: level of