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Experimental paper

Influence of argon on temperature modulation and neurological outcome in hypothermia treated rats following cardiac arrest

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ABSTRACT

Aim of the study: Combining xenon and mild therapeutic hypothermia (MTH) after cardiac arrest (CA) confers a degree of protection that is greater than either of the two interventions alone. However, xenon is very costly which might preclude a widespread use. We investigated whether the inexpensive gas argon would enhance hypothermia induced neurologic recovery in a similar manner.
Methods: Following nine minutes of CA and three minutes of cardiopulmonary resuscitation 21 male Sprague-Dawley rats were randomized to receive MTH (33 °C for 6 h), MTH plus argon (70% for 1 h), or no treatment. A first day condition score assessed behaviour, motor activity and overall condition. A neurological deficit score (NDS) was calculated daily for seven days following the experiment before the animals were killed and the brains harvested for histopathological analysis.
Results: All animals survived. Animals that received MTH alone showed best overall neurologic function. Strikingly, this effect was abolished in the argon-augmented MTH group, where animals showed worse neurologic outcome being significant in the first day condition score and on day one to three and five in the NDS in comparison to MTH treated rats. Results were reflected by the neurohistopathological analysis.
Conclusion: Our study demonstrates that argon augmented MTH does not improve functional recovery after CA in rats, but may even worsen neurologic function in this model.

Introduction

Despite improvements in post resuscitation care in recent years, many survivors of out-of-hospital cardiac arrest (OHCA) die subsequently while hospitalized. Among these, neurologic deficits are common [1,2]. The high mortality rate is attributable to the severity of the post cardiac arrest syndrome, which includes coagulation abnormalities, inflammation and endothelial activation [3]. The resulting degree of neurological and cardiac dysfunction, rarely multi-organ failure, ultimately represents the limiting factor for overall survival in OHCA patients. Mild therapeutic hypothermia (MTH) is the only proven treatment to reduce neurological sequelae and mortality after cardiac arrest (CA) [4]. However, Kim and colleagues showed in 2013 that aggressive prehospital cooling does not improve survival or neurological status [5]. In addition, MTH did not have a significant advantage over the prevention of fever in a European multicentre study in patients with OHCA [6]. And although MTH appears to be effective in improving neurological outcome, benefit is often insufficient and additional therapeutic strategies are needed [5,6]. Thus, controversial discussion arises on the optimal temperature management after CA.

Noble gas mediated neuroprotection has gained considerable attention in the last decade and especially xenon has been extensively studied in several models of ischemia/reperfusion injury [7–10]. Several groups published data suggesting that these neuroprotective properties are present in large animal models of CA [11–13]. Recent data, especially in models of neonatal asphyxia [14,15], revealed that the combination of MTH and xenon confers an additive neuroprotective effect exceeding the effects of MTH alone. Given the negligible side effects of the gas, which are well characterized from its use as an anaesthetic [16], these remark-

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able results have led to phase II clinical trials, exploring the effects of xenon as an adjunct to MTH. First results revealed the feasibility and cardiac safety of xenon combined with MTH in survivors of OHCA [17]. However, this treatment combination did not significantly exceed the effect of hypothermia alone in outcomes or mortality at six months [18]. Irrespective of the results of further investigations, it has to be noted that xenon is quite expensive and its delivery requires closed circuit anaesthesia machines to administer the gas in an economical fashion. These aspects might preclude xenon from a widespread clinical use.

Argon is much more abundant and available at a significantly lower cost. Interestingly, there is mounting evidence that argon, albeit lacking narcotic effects, has organ-protective properties [19–27]. Additionally, first results from two preclinical studies revealed that argon augmented hypothermic protection in the setting of neonatal hypoxic ischemic encephalopathy in pigs and rats [28,29]. Hence, this study was designed to investigate whether argon would enhance hypothermia induced neurological recovery in a rodent model of cardiac arrest induced neurological sequelae.

Methods

Experiments were performed in 21 male Sprague-Dawley rats (Charles River, Germany) weighing between 400 and 500 g. We tested the neuroprotective potency of 70% argon augmented MTH (Argon + MTH) in comparison to MTH (MTH) alone or no treatment (Control). Argon gas was administered using custom-made gas cylinders containing the desired concentration (Linde Gas Therapeutics, Unterschleißheim, Germany). All animals were treated according to the following protocol unless mentioned otherwise.

Animals were housed in adequately spaced cages (60 cm × 40 cm; type 2000; Tecniplast, Buguggiate, Italy) with a 12-h light-dark cycle. 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 carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals formulated by the National Research Council (National Academies Press, 1996) and the ARRIVE guidelines (National Centre for the Replacement, Refinement and Reduction of animals in research, 2010). In addition, all reported data and outcomes are in accordance with the Utstein style guidelines for uniform reporting of laboratory cardiopulmonary resuscitation (CPR) research [30].

Animal preparation

We used a rat model of CA and CPR as previously described [23]. Anaesthesia was induced 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, respiratory frequency, or movements of the tail or paws. Chest and back were shaved to allow for direct contact of the paddles used for defibrillation during CPR.

On a surgical board in supine position, the trachea was orally intubated using a modified 14 G cannula (Abbocath-T, Abbott Hospital Division, North Chicago, IL, USA). Animals were mechanically ventilated (Servo Ventilator 900C, Siemens ELEMA, Munich, Germany) 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 analyser (Cap Star 100, CWE Inc., Ardmore, PA, USA). A 3 lead electrocardiogram was continuously registered and recorded *via* monopolar needle electrodes (MLA1204 Needle Electrodes, ADinstuments, Oxford, UK). The right femoral artery was surgically exposed, cannulated with a polyethylene catheter (PE 50) and connected to a high sensitivity transducer (Capto SP 844 Physiologic Pressure Transducer, Capto Inc., Skoppum, Norway) for the measurement of mean arterial pressures (MAP), respectively. A thermocouple microprobe (IT-18, Physitemp Instruments, Clifton, NJ, USA) was placed into the oesophagus. The temperature was monitored and maintained between 37 and 37.5 °C using a heating mat (TCAT-2LV-controller, Physitemp Science Products, Hofheim, Germany). The left femoral vein was also cannulated with an additional PE 50 catheter to allow for administration of fluids and epinephrine during CPR. Catheters were flushed intermittently with saline solution containing 2 IU ml^{-1} of heparin.

Experimental procedure

An established model of electrically induced cardiac arrest and mechanical resuscitation was employed in this investigation [23]. Ventricular fibrillation (VF) was induced by transoesophageal electrical stimulation. After fluoroscopy guided placement of the electrode, 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 nine minutes 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} adrenaline (epinephrine) was administered via the femoral line 30 s after starting chest compressions. After three minutes of CPR, external defibrillation with 5 J (Zoll MSeries, Zoll Medical Corporation, Chelmsford, MA, USA) was attempted up to three times. If a return of spontaneous circulation (ROSC) was not achieved, chest compressions and administration of adrenaline at the same dosage were repeated for one minute before additional direct current counter-shocks were delivered. This cycle was repeated up to three times. ROSC was confirmed by organized electric activity in conjunction with a rise in MAP to greater than 50 mmHg. After restoration of spontaneous circulation, animals were randomized into three groups using the sealed envelope method. All groups received 100% oxygen for the first hour following ROSC. One hour after successful resuscitation, FiO_2 was reduced to 0.3. Animals of the MTH-group (n = 7) were cooled immediately after ROSC for six hours and reached the target temperature of 32–34 °C within 15 min. Gradual rewarming was achieved within one hour using a heating pad. Animals of the Argon + MTH group (n = 7) were treated identically and additionally received 70% argon ventilation for one hour, one hour after ROSC, as neuroprotective effectiveness of this Argon treatment has been proven in our previously published work [23]. The control group (n = 7) did not receive any additional treatment and was kept normothermic. Before animals were weaned from the ventilator and extubated seven hours post ROSC, a temperature transmitter (TA-F40, Data science international, Minnesota, USA) was implanted intraabdominally, to provide for a 17 h post-extubation temperature measurement. All animals received a single subcutaneous injection of 0.1 mg kg^{-1} buprenorphine for pain relief. Following extubation, animals were observed for approximately 30 min to ensure adequate spontaneous breathing before being returned to their cages. For the first 17 h post extubation, cages were placed on a recording platform for body temperature readings (Data exchange matrix/Dataquest A.R.T. Software, Data science international, MN, USA); room temperature was kept between 20 and 21 °C.