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Recover of peripheral nerve function after prolong hypothermic cardiac arrest in a porcine model with extra corporeal life support

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ABSTRACT

Objectives: Surviving long lasting cardiac arrest following accidental hypothermia has been reported after treatment with extra corporeal life support (ECLS), but there is a risk of neurologic injury. Most surviving hypothermia patients have a prolonged stay in the intensive care unit, where most patients experience polyneuropathy. Theoretically, accidental hypothermic cardiac arrest may in itself contribute to polyneuropathy. This study was designed to examine the impact of three hours of cardiac arrest at a core temperature of 20 °C followed by reanimation of peripheral nerve function.

Methods: Seven pigs were cannulated for ECLS and cooled to a core temperature of 20 °C followed by three hours of circulatory arrest where the extremities were packed with ice. After three hours, ECLS was started and rewarming was performed. During the process, neural testing of the ulnar nerve (a somatic nerve) and of the vagus nerve (an autonomic nerve) were performed and blood was drawn for analysis of p-potassium, serum-neuron-specific enolase, and S100b protein.

Results: The ulnar nerve was cooled from 34.9 ± 1.6 °C to 12.8 ± 3.8 °C and the vagus nerve from 36.2 ± 1.2 °C to 15.4 ± 1.4 °C. Physiologic function of both somatic and autonomic nerves were strongly affected by cooling, but recovered to almost normal levels during rewarming, even after three hours of hypothermic cardiac arrest. P-potassium rose from 3.9 (3.6–4.6) mmol/l to 8.1 (7.2–9.1) mmol/l after three hours of cardiac arrest, but normalized after recirculation. There was no rise in serum-neuron-specific enolase, but a slight rise in S100b protein during three hours of hypothermic cardiac arrest was observed. All pigs obtained return of spontaneous circulation (ROSC).

Conclusions: Reanimation after three hours of hypothermic cardiac arrest using ECLS was possible with no or, if present, minor damage to the two nerves tested.

1. Introduction

During recent decades, technical improvements have made the use of extracorporeal life support (ECLS) widespread in the treatment of deep hypothermic circulatory arrest (CA). Many striking cases have been reported with long lasting CA and temperatures as low as 13.7 °C (Gilbert et al., 2000; Walpoth et al., 1997). In Denmark, there was a boating accident in 2011 with seven teenagers resuscitated from drowning with deep hypothermic circulatory arrest, probably for several hours at a temperature of approximately 20 °C (Wanscher et al., 2012). We rarely know the exact time of CA nor the level of anoxia and temperature before CA. From cardiac surgery it is well known that a controlled cardiac arrest at 20 °C has a safe window of up to approximately 60 min with no important neurological damage (Kirklin and Barratt-Boyes, 2013). We do not know the limits for resuscitating a deep hypothermic victim in an acceptable neurologic condition, but retrospectively a p-potassium exceeding 10 mmol/l is a surrogate of anoxic damages with no chance of saving the victim (Schaller et al., 1990). In a pig model we demonstrated a rise in p-potassium to 10 mmol/l in approximately 3½ h with CA at 20 °C, but the neurological condition of the animals after the hypothermic CA was not investigated (Kjaergaard et al., 2010). For most patients surviving

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prolonged hypothermic cardiac arrest, a long stay in the intensive care unit can be expected, and polyneuropathy and myopathy are observed in the majority of patients even though they did not have cardiac arrest before hospitalization (Pati et al., 2008; Zhou et al., 2014). Many attempts have been made to assess brain damage early in comatose patients surviving cardiac arrest, but the risk of peripheral nerve injuries due to prolonged hypothermic arrest is not well described. The majority of the attention in this area has been focused on brain damage and to some extent neuron-specific enolase, which has been a predictor for poor outcomes due to brain damage, but it is not very applicable in the first 12 h after cardiac arrest (Rech et al., 2006). In a pig model of hypothermic cardiac arrest for 30 min, no difference in neuron-specific enolase was detected (Debaty et al., 2016). A presumably better early indicator of brain injuries seems to be glial protein S100b; a protein that normally does not penetrate the blood-brain barrier, unless there is a brain injury (Bottiger et al., 2001; Hansen-Schwartz and Bouchelouche, 2014; Stammet et al., 2015).

If a victim survives hypothermic cardiac arrest, not only brain injuries and polyneuropathy threaten recovery. The immune and nervous systems interact in many ways as a response to stress or injury, and one of the clinical problems after hypothermic cardiac arrest is kidney dysfunction (Walpoth et al., 1997). We do not know how much influence the vagus nerve has on this, but vagus nerve stimulation has been shown to protect against ischaemia-reperfusion injury in a mouse model (Inoue et al., 2016).

Many experiments have been performed with isolated nerves with anoxia and different temperatures, showing that hypothermia acts as a protectant against anoxia (Stecker et al., 2013). However, in a whole animal model with resuscitation there will be the influence of reperfusion and changes in blood biochemistry in the re-animation period. There has been considerable attention on reperfusion injuries during the last few years, and it is suspected that reperfusion per se can result in injury (Bagdatoglu et al., 2008; Eltzschig and Collard, 2004; Mitsui et al., 1999; Nukada et al., 1997).

The influence on peripheral nerves, somatic or autonomic, from prolonged hypothermic cardiac arrest and reanimation is not very well elucidated. We here present results from an experimental study with cardiac arrest at 20 °C for three hours in a pig model. The present analysis investigated the functional recovery of the somatic ulnar nerve and the autonomic vagus nerve after that interval.

2. Material and methods

Seven female Danish Landrace pigs ~ 35 kg were used for the experiments. The study was approved by the Animal Experiments Inspectorate (no 2014-15-2934-01001), and the methods were in line with the Utstein recommendation for uniformity in animal experiments (Idris et al., 1996). The animals were housed at 23 °C, room temperature, and fasted from the morning before anaesthesia, but they had access to water.

2.1. Medication

The anaesthesia was induced with Zoletil® (a mixture of two dissociative anaesthetics (Ketamin 6.25 mg/ml and Tiletamin 6.25 mg/ml), a benzodiazepine (Zolazepam 6.25 mg/ml), a synthetic opioid (Butorphanol 1.25 mg/ml) and Xylazin (6.5 mg/ml) an alpha 2 adrenergic agonist, which contains both sedative, hypnotic, analgesic and muscle relaxing properties). Anaesthesia was maintained with continuous intravenous infusions of Propofol, Midazolam and Fentanyl based on the clinical demand as reflected in haemodynamic changes during hypothermia and/or movement of the pig. Bradycardia was treated with intravenous Atropine 0.5 mg. During the three hours of circulatory arrest no medications were given.

When weaning from extra corporeal circulation (ECC), norepinephrine was administered in small doses if needed. In the case of profound hypoglycaemia below 2.5 mmol/l, an intravenous dose of 10 mmol glucose was administered.

2.2. Animal surgery

The trachea was intubated with a 6.5 mm cuffed endotracheal tube (Portex Blue line, Smiths medical, UK) and the lungs were ventilated with a ventilator (Dameca DREAM, Roedovre, Denmark) using volume controlled positive pressure ventilation (PEEP: 5 cm H_2O; V_t: 8–10 ml/kg; respiratory frequency: 10–20/minute, adjusted according to P_aCO_2 inspiratory/expiratory ratio: 1:1). A 5 French catheter was inserted into the femoral artery for blood pressure monitoring and sampling of blood. Continuous blood pressure and ECG were monitored using Datex-Ohmeda S/5 (GE Healthcare, Broendby, Denmark).

A vein catheter was inserted into a femoral vein for fluid and drug infusions. A bladder catheter with a temperature gauge (Smiths Medical ASD Inc., Rockland, Massachusetts, USA) was inserted for continuous temperature monitoring and measurements of diuresis.

The skin was incised on both sides of the neck for access to the right jugular vein and the left vagus nerve. The skin over the left foreleg was incised, giving access to the ulnar nerve.

After sternotomy, another temperature gauge was inserted in the abdominal cavity via a small opening in the diaphragm.

2.3. Extra corporeal circulation

After sternotomy, an extracorporeal circuit was established between a 29 Fr three stage catheter (Medtronic, Minneapolis, MN, USA) inserted via the right jugular vein into the caval vein and a 19 Fr catheter (Medtronic, Minneapolis, MN, USA) inserted into the ascending aorta. The circuit contained a centrifugal pump (Jostra Rotaflow, Maquet Cardiopulmonary AG, Hirrlingen, Germany) and an oxygenator with a heat exchanger (Jostra Rotaflow, Maquet Cardiopulmonary AG, Hirrlingen, Germany). The system was primed using normal saline. The animals were treated with heparin to obtain an activated coagulation time of more than 800 s (Haemochron 301, International Technidyne Corporation, Piscataway, New Jersey, USA). During the whole experiment gas exchange via the lungs and via the oxygenator was adjusted according to the results of arterial blood tests.

2.4. Neural testing

2.4.1. Electrodes

An incision was made on the inside of the left forelimb and a 10–15 cm segment of the ulnar nerve was isolated from the surrounding tissues. Two tripolar cuff electrodes (2.4 mm inner diameter, 5 mm centre-to-centre electrode distance, ~12 mm total length) were then mounted on the nerve with 55 ± 7 (mean ± standard deviation) mm between the centres of each cuff electrode. Another incision was made on the left side of the trachea and a ~15 cm segment of the left vagus nerve was isolated from the surrounding tissues. Two tripolar cuff electrodes (2.4 mm inner diameter, 10 mm centre-to-centre electrode distance, ~22 mm total length) were mounted on the nerve with 72 ± 14 mm between the centres of each cuff. All cuff electrodes used in the experiment consisted of a medical grade silicone cuff with three one mm wide platinum rings embedded on the inside and were produced according to the technique described by Haugland (1996). After placement of the cuff electrodes, the distances between the centre electrodes of the stimulation and recording cuffs on the ulnar and vagus nerves, respectively, were measured to enable calculation of the nerve conduction velocity.

2.4.2. Electrical stimulation

Nerve recruitment curves were obtained by stimulation applied to the nerves using the proximal cuff electrodes. Bi-phasic constant-current pulses with a primary (cathodic) phase of 100 μs duration