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Effects of adenosine monophosphate on induction of therapeutic hypothermia and neuronal damage after cardiopulmonary resuscitation in rats☆

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ABSTRACT

Background: Animal studies and pathophysiological considerations suggest that therapeutic hypothermia after cardiopulmonary resuscitation is the more effective the earlier it is induced. Therefore this study is sought to examine whether pharmacological facilitated hypothermia by administration of 5′-adenosine monophosphate (AMP) is neuroprotective in a rat model of cardiac arrest (CA) and resuscitation.
Methods: Sixty-one rats were subjected to CA. After 6 min of ventricular fibrillation advanced cardiac life support was started. After successful return of spontaneous circulation (ROSC, $n = 40$), animals were randomized either to placebo group ($n = 14$) or AMP group (800 mg/kg body weight, $n = 14$). Animals were kept at an ambient temperature of 18 °C for 12 h after ROSC and core body temperature was measured using a telemetry temperature probe. Neuronal damage was analyzed by counting Nissl-positive (i.e. viable) neurons and TUNEL-positive (i.e. apoptotic) cells in coronal brain sections 7 days after ROSC. Functional status evaluated on days 1, 3 and 7 after ROSC by a tape removal test.
Results: Time until core body temperature dropped to <34.0 °C was 31 min [28; 45] in AMP-treated animals and 125 min [90; 180] in the control group ($p = 0.003$). Survival until 7 days after ROSC was comparable in both groups. Also number of Nissl-positive cells (AMP: 1 [1; 7] vs. placebo: 2 [1; 3] per 100 pixel; $p = 0.66$) and TUNEL-positive cells (AMP: 56 [44; 72] vs. placebo: 53 [41; 67] per 100 pixel; $p = 0.70$) did not differ. Neither did AMP affect functional neurological outcome up to 7 days after ROSC. Mean arterial pressure 20 min after ROSC was 49 [45; 55] mmHg in the AMP group in comparison to 91 [83; 95] mmHg in the control group ($p < 0.001$).
Conclusion: Although application of AMP reduced the time to reach a core body temperature of <34 °C neither survival was improved nor neuronal damage attenuated. Reason for this is probably induction of marked hypotension as an adverse reaction to AMP treatment.

1. Introduction

In the United States and Europe approximately 1 million patients suffer from out-of-hospital cardiac arrest (CA). Although initially in 25–50% a restoration of spontaneous circulation (ROSC) can be achieved, only 2–10% survive without major neurological deficit.[1–3] Guidelines of the European Resuscitation Council and the American Heart Association mention therapeutic hypothermia of 32.0–34.0 °C after CA as level I recommendation.[4,5] Numbers needed to treat with therapeutic hypothermia were 6 to save 1 life, and 5 patients to improve neurological outcome.[6,7]

Animal experiments suggest that the faster hypothermia is inducted the more effective it is.[8,9] Clinical data display a less clear picture. There were two retrospective analyses in the past years with contradictory results.[10,11] Just recently the prospective randomized trial by Kim et al.[12] has shown no benefit for the patients with prehospital cooling after ROSC, although the time to reach targeted temperature of <34.0 °C was reduced by more than

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1 h. Possibly infusion of up to 2 l of normal saline increased the incidence of rearrests and pulmonary oedema and was therefore causative for the neutral outcome.

Other cooling devices have the drawbacks of a substantial degree of invasiveness and a considerable delay in inducing hypothermia.[13,14] Reasons for this delay are on the one hand the expenditure of time until insertion of an endovascular cooling catheter, e.g., and on the other hand physiological counter-regulation (vasoconstriction, shivering) of the patient. Therefore pharmacological facilitation of hypothermia might be desirable ("facilitated hypothermia").[15]

Administration of the endogenous nucleotide 5′-adenosine monophosphate (AMP) induces profound hypothermia in rodents.[16–18] Therefore we hypothesized that AMP can accelerate induction of therapeutic hypothermia under otherwise identical conditions. Thereby this might improve neurological outcome in a rat model of CA.

2. Materials and methods

Healthy adult male Wistar rats (Janvier, France) were obtained at 6 weeks of age and maintained in our animal colony at 12 h light:12 h dark circadian rhythm and 22 °C for 2–3 weeks prior to experimentation. Two animals were housed per cage on cottonwood bedding (PK-3, LASvendi, Germany) and given food (ROD 16A, LASvendi, Germany) and water ad libitum.

The trial was investigator blinded, randomized, and placebo controlled. After institutional approval by the Governmental Animal Care Committee a total number of 63 rats (330 [299; 340] g body weight) were consecutively allocated either to placebo or interventional group ($n = 14$ each group) by a random sequence generator (http://www.agitos.de/zufallsgenerator.html). All animals were handled in accordance with the European Communities Council Directive of November 24th, 1986 (86/609/EEC).

2.1. Animal preparation and experimental protocol

Under general anaesthesia (sevoflorane concentration initially 3–4%, decreasing to 1–2% according to the required anaesthesia depth; N_2O/O_2 70:30), the trachea of the animals was incubated and animals were mechanically ventilated (Harvard Model 683 Small Animal Ventilator, Harvard Apparatus, Massachusetts) with a tidal volume of 8 ml/kg bodyweight. Breathing rate was approximately 60/min and adapted to maintain normocapnia (assessed by blood gas analyses), no positive end-expiratory pressure. Polyethylene catheters were inserted into the left femoral artery and vein. Telemetry temperature probes (VitalView Series 4000 G2 E-Mitter, Mini Mitter, Bend, OR) for continuous temperature control were surgically inserted into the rats' abdominal cavities. Arterial blood pressure in the abdominal aorta was measured continuously, an electrocardiogram was recorded by two subcutaneous needle electrodes, and the tympanic temperature was registered and kept within a range of 36.9–37.8 °C by a heating plate (Hot Plates Typ 062, Labotect, Germany) where the animals were placed on.

Ventricular fibrillation was initiated using alternating current (12 V, 50 Hz) via an oesophageal electrode and ventilation was stopped. After 6 min of CA CPR was started: 60 breaths/min (100% oxygen), external manual chest compression at a rate of 200/min, duty cycle 50%, and compression depth of 25% of the anterior–posterior diameter of the chest. Adrenaline (epinephrine 20 μg/kg) was administered i.v. immediately. After 2 min of CPR without ROSC a defibrillation attempt was performed with one biphasic shock of 1 J (M-Series, Zoll Corporation, Germany). CPR was continued and adrenaline administered repeatedly, if ROSC could not be achieved 30 s after the first defibrillation attempt. Defibrillation procedures were repeated after 30 s each. For buffering after 4 min of CPR 0.2 ml sodium bicarbonate (8.4%) was administered. In case of 6 min of unsuccessful CPR, resuscitation was stopped and the animal was declared dead. ROSC was defined as maintenance of an unassisted MAP beyond values of 50 mmHg for at least 10 consecutive minutes according to the Utstein-style guidelines.[19] Once ROSC has been achieved, no further steps like defibrillation attempts, cardioversion, and administration of vasopressors or antiarrhythmic drugs were taken.

Fifteen minutes after ROSC 800 mg/kg body weight AMP (A1752, Sigma–Aldrich, Germany) in 2.5 ml of 0.9% saline solution or placebo (2.5 ml of 0.9% saline solution) was administered intraperitoneally. Breathing rate was adjusted to reach normocapnia 20 min after ROSC and maintain it until sufficient spontaneous ventilation and extubation.

Each catheter insertion site was infiltrated with 0.5 ml of lidocaine 2%. 35 min after ROSC all catheters were removed and animals were transferred to a cooling chamber with an ambient temperature of 18.0 °C for 12 h. Core body temperature was registered every minute. Rewarming was passive by moving the animals to an ambient temperature of 22 °C. Seven days after ROSC animals were euthanized by decapitation in deep anaesthesia.

2.2. Tape removal test

To evaluate the neurological state in all successfully resuscitated animals, the tape removal test (TRT) was assessed at 1, 3 and 7 days after cardiac arrest as described previously.[20] In brief, self-adhesive tapes were affixed to each of the animal's front paws in the distal region of the wrist. The period until removal of the first piece of tape by the animal using its teeth was recorded in order to quantify sensorimotor neurological damage. The test was truncated at 60 s.

2.3. Morphology

To evaluate neuronal degeneration in resuscitated animals, slices of the hippocampal CA-1 sector were examined histologically. For in situ staining of DNA fragmentation and apoptotic bodies, the histochemical TUNEL method was used. The hippocampal CA-1 sector was analyzed by counting all TUNEL-positive cells at the magnification of 400-fold and divided by the according length of the entire CA-1 sector (number of TUNEL-positive cells/100 pixel). According to the same method, histological examination was performed on Nissl-stained 10 μm sections at the level of the dorsal hippocampus (number of Nissl-positive, i.e. viable neurons/100 pixel).

2.3.1. Sample size calculation and statistical analysis

Sample size was calculated according to earlier data from TUNEL and Nissl-staining. To detect a difference in viable and TUNEL-positive cells of about 25% with a power of 80%, 14 animals per group were needed. Only data obtained from animals surviving to the predefined reperfusion endpoint were analyzed. Enrolment and randomization was therefore performed until a total of 14 animals per group was reached.

Primary target parameters were core body temperature, number of Nissl- and number of TUNEL-positive neurons. Secondary outcome parameters were survival and functional neuronal deficit as assessed by the TRT.

Core body temperature for the respective group was averaged for each minute after ROSC and is presented as median [quartile 1; quartile 3]. Data from the TRT, physiological variables and histopathological cell counts are also shown as median [quartile 1; quartile 3]. For statistical analysis a Wilcoxon test was employed.