Resuscitation 92 (2015) 26–31

Contents lists available at ScienceDirect

Resuscitation

journal homepage: www.elsevier.com/locate/resuscitation

Experimental paper

Effect of a pharmacologically induced decrease in core temperature in rats resuscitated from cardiac arrest☆

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ARTICLE INFO

Article history:
Received 8 October 2014
Received in revised form 1 April 2015
Accepted 11 April 2015

Keywords:
Cardiac arrest
Resuscitation
Hypothermia

ABSTRACT

Aim: Hypothermia is recommended by international guidelines for treatment of unconscious survivors of cardiac arrest to improve neurologic outcomes. However, temperature management is often underutilized because it may be difficult to implement. The present study evaluated the efficacy of pharmacologically induced hypothermia on survival and neurological outcome in rats resuscitated from cardiac arrest.

Methods: Cardiac arrest was induced for 10 min in 120 rats. Sixty-one rats were resuscitated and randomized to normothermia, physical cooling or pharmacological hypothermia 5 min after resuscitation. Pharmacological hypothermia rats received a combination of ethanol, vasopressin and lidocaine (HBN-1). Physical hypothermia rats were cooled with intravenous iced saline and cooling pads. Rats in the pharmacological hypothermia group received HBN-1 at ambient temperature (20 °C). Normothermic rats were maintained at 37.3 ± 0.2 °C.

Results: HBN-1 ($p < 0.0001$) shortened the time (85 ± 71 min) to target temperature (33.5 °C) versus physical hypothermia (247 ± 142 min). The duration of hypothermia was 17.0 ± 6.8 h in the HBN-1 group and 17.3 ± 7.5 h in the physical hypothermia group ($p = 0.918$). Survival ($p = 0.034$), neurological deficit scores ($p < 0.0001$) and Morris Water Maze performance after resuscitation ($p = 0.041$) was improved in the HBN-1 versus the normothermic group. HBN-1 improved survival and early neurological outcome compared to the physical hypothermia group while there was no significant difference in performance in the Morris water maze.

Conclusion: HBN-1 induced rapid and prolonged hypothermia improved survival with good neurological outcomes after cardiac arrest suggesting that pharmacologically induced regulated hypothermia may provide a practical alternative to physical cooling.

1. Introduction

Targeted temperature management is recommended to reduce brain damage after resuscitation from cardiac arrest in humans although the optimal target temperature remains controversial.[1–4] The American Heart Association (AHA) and the International Liaison Committee on Resuscitation include hypothermia in their guidelines for treatment of patients resuscitated from cardiac arrest. Despite these guidelines, therapeutic hypothermia is underutilized with less than 12% of hospitals in the US using this potentially lifesaving approach.[5,6] The most often cited reason for not using therapeutic hypothermia is "it is too technically difficult."[5]

Physical methods to forcefully lower body temperature include ice bags, cooling pads and endovascular devices. Such cooling methods can be inefficient in lowering core body temperature of adult subjects because of naturally occurring thermoregulatory responses consisting of shivering, cutaneous vasoconstriction and increased metabolism.[7] As a result, drugs such as opiates and paralytics are often given before initiation of physical cooling methods.[8]

☆ A Spanish translated version of the abstract of this article appears as Appendix in the final online version at http://dx.doi.org/10.1016/j.resuscitation.2015.04.009.

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http://dx.doi.org/10.1016/j.resuscitation.2015.04.009
0300-9572/

Furthermore, these drugs add to the complexity and safety of cooling because they may induce hypotension, altered mental status, respiratory arrest, and the need for mechanical ventilation. In addition, the equipment and drugs required for induction of physical hypothermia are not always available pre-hospital, thus delaying the initiation of cooling and time to target temperature which may influence neurological outcome.[9]

HBN-1 was developed as a pharmacological alternative to physical methods to lower core temperature and induce a regulated state of therapeutic hypothermia.[10,11] Regulated hypothermia is thought to be operative by lowering the body's temperature set point in the hypothalamic regulatory center.[12] When the temperature set point is lowered, the body responds by reducing metabolic rate, blocks shivering, and increases heat loss through peripheral vasodilatation and sweating. HBN-1 is a patented pharmaceutical preparation that combines ethanol, vasopressin and lidocaine.[10] It induces a rapid and prolonged hypothermia even at room temperature without the need for chemical paralysis, sedation, ancillary equipment, or mechanical ventilation.[10] The drug is administered intravenously so paramedics could use it in the field, thus minimizing delays in inducing hypothermia.

The purpose of this study was to evaluate the effect of HBN-1 on body temperature, mortality and neurological outcome in rats resuscitated from cardiac arrest. We hypothesized that survival with good neurological outcome following pharmacologically induced hypothermia would be significantly different compared to normothermia or physically induced cooling.

2. Materials and methods

The Institutional Animal Care and Use Committee approved the study in accord with National Institutes of Health Guidelines. Sprague-Dawley female rats weighing 300–350 g were resuscitated from asphyxial cardiac arrest and then randomized to normothermic, physical hypothermic or pharmacological hypothermic (HBN-1) groups. We prospectively assigned a treatment group to rats after resuscitation from cardiac arrest by picking a piece of paper (12 with normothermia, 12 with physical hypothermia and 12 with HBN-1) out of an opaque jar with the group assignment on the paper. If the rat survived 15 days, that assignment would be removed from further assignment. If the rat did not survive the 15 days, the data from that rat would be analyzed, but the assignment was placed back in the jar to assure that 12 rats would be available for Morris Water Maze analysis in each group upon completion of the study. Rats in the normothermic group had their core body temperature maintained at a target temperature of 37.3 °C before asphyxial cardiac arrest and during reperfusion. Rats in the physical hypothermic group were wrapped in a cooling pad set to 4 °C (Medivance, Arctic Sun, Louisville, CO) and infused with iced saline (4 °C) starting 5 min after return of spontaneous circulation (ROSC) and continued for 12 h after resuscitation. Hypothermia was maintained in the physical hypothermic group by having the core temperature signal from a surgically implanted telemetric probe (MiniMitter, Bend, OR) control a servo-regulated incubator (Brinsea, Titusville, FL), the cooling pad, and a fan. Rats in the pharmacological hypothermic group received room temperature HBN-1 (ethanol 3.03 g/kg, vasopressin 0.13 U/kg, lidocaine 3.2 mg/kg) infused intravenously at ambient temperature (20 °C) starting 5 min after ROSC and continued for 12 h after resuscitation. All three groups received an initial 30 ml/kg bolus of fluid (normal saline at room temperature in the normothermic group and normal saline at 4 °C in the physical hypothermic group) initiated 5 min after ROSC at a rate of 60 ml/kg/h × 30 min followed by 1.5 ml/kg/h for 12 h. The time to target temperature was defined as the time from initiation of cooling until a core temperature of 33.5 °C was attained. All rats were prepared for asphyxial cardiac arrest and reperfusion as previously described.[13] Briefly, rats were anesthetized with 4% isoflurane, intubated and mechanically ventilated with a combination of 30% oxygen and 70% nitrous oxide and wrapped in a thermal blanket to approximate the mass and thermal inertia of a larger mammal.[14] Rats were then covered with Arctic Sun pads (turned off in the normothermic and HBN-1 groups), and titrated isoflurane anesthesia was maintained throughout the surgery. Catheters were placed in a femoral vessel to monitor mean arterial blood pressure, for blood draws, and for administration of intravenous drugs. Blood samples and cardiovascular parameters were recorded at baseline and at 10 min, at 30 min, and at 30 min intervals thereafter, until 180 min after ROSC. A telemetric temperature probe (Data Sciences International, St. Paul, MN) was inserted and secured to the posterior peritoneum, behind the liver via a midline laparotomy. Rats were chemically paralyzed with vecuronium (1 mg/kg) intravenously, and apneic asphyxia was induced by interrupting ventilation. Asphyxia led to cardiac arrest within 4 min in all rats, and asphyxia was maintained for 10 min. Rats were resuscitated with intravenous epinephrine (0.008 mg/kg), sodium bicarbonate (1 mEq/kg), mechanical ventilation with 100% oxygen and chest compressions. Chest compressions were stopped when there was ROSC (mean arterial pressure greater than 60 mmHg for more than 5 min) or no ROSC after 2 min. Rats were extubated 180 min after ROSC, the arterial line was removed and the venous line was tunneled through to the shoulder, externalized and connected to a tether which allowed the rats unrestricted movement. Rats had free access to food and water during recovery. A neurological deficit score was performed daily for 10 days after ROSC by an investigator blinded to therapeutic interventions. The rat neurological deficit score tested cranial nerve function, coordination (balance beam walk, placing test, depth perception, righting reflex), motor and sensory function.[13] On days 11–15 after ROSC, rats were trained in a Morris Water Maze with four swimming sessions a day by a research assistant blinded to intervention. The rats were placed in one of four entrance quadrants (north, south, east or west) of the pool in random order and swam until they found the hidden platform or 90 s had elapsed without finding the platform. If a rat was unable to find the hidden platform, they were placed on the hidden platform for 30 s. Rats learn to find the hidden platform by referencing the location of illuminated figures on the side of the pool relative to the hidden platform.[15] The average time over four swimming trials required to locate the hidden platform (latency time) was compared between groups. After completion of Morris Water Maze testing rats were euthanized with an overdose of isoflurane.

2.1. Statistical analysis

Physiological variables, neurological deficit scores and latency time were reported as means and standard deviations and compared between groups at specific time point(s) using analysis of variance. Latency time across days was also compared between groups by a repeated measure ANOVA. Kaplan–Meier curves were used to evaluate survival in the groups and the difference was compared using a log rank test. p-Values ≤ 0.05 were considered statistically significant. All statistical analysis was performed using SAS v 9.3 (SAS Institute, Cary, NC).

3. Results

Fifty-one percent of 120 rats were successfully resuscitated following induction of asphyxial cardiac arrest. Physiological variables at baseline were comparable between groups. There was no difference in time to cardiac arrest or ROSC between groups. The time from ROSC to a target temperature (TTT) of 33.5 °C was shorter