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EXPERIMENTAL PAPER

Protein nitration and poly-ADP-ribosylation in brain after rapid exsanguination cardiac arrest in a rat model of emergency preservation and resuscitation☆

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Summary
Background: Emergency preservation and resuscitation (EPR) of 60 min in rats is achievable with favorable outcome, while 75 min is associated with substantial mortality and impaired neurological outcome in survivors. We hypothesized that 75 min but not 60 min of EPR would be associated with activation of two potential secondary injury cascades in brain as reflected by protein nitration and poly (ADP-ribose) polymerase (PARP) activation.
Methods: Rats were rapidly exsanguinated over 5 min. After 1 min of cardiac arrest (CA), rats were cooled to a target tympanic temperature of 15 °C. After either 60 min or 75 min of CA, resuscitation was achieved via cardiopulmonary bypass (CPB). Rats subjected to CPB only served as controls. Overall performance category (OPC) and neurologic deficit score (NDS) were assessed at 24 h. Protein nitration and poly-ADP-ribosylation were assessed by Western blotting and immunohistochemistry for 3-nitrotyrosine and poly-ADP ribose polymers, respectively, in multiple brain regions.
Results: Neurologic outcome was better in the 60 min vs. the 75 min EPR group (OPC, $P < 0.001$; NDS, $P = 0.001$). Densitometric analysis of the major 64 kD band showed that nitration and PARP activation were significantly increased in hippocampus, cortex and striatum in the 75 min EPR

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group vs. other groups. However, there were no differences in cerebellum. Analysis of the full protein spectrum showed significantly increased PARP activation only in hippocampus in the 75 min EPR group vs. other groups.
Conclusions: Extending the duration of EPR beyond the limit that can yield favorable recovery in rats was associated with increased nitration and ribosylation of selected proteins in selectively vulnerable brain regions. The impact of these mechanisms on the outcome remains to be determined.

Introduction

Emergency preservation and resuscitation (EPR) is a novel concept initially developed to treat cardiac arrest (CA) after trauma and rapid exsanguination.[1] It utilizes a strategy to preserve the viability of organs and organism using hypothermia during an otherwise lethal insult to buy time for transport and surgical repair followed by delayed resuscitation with cardiopulmonary bypass (CPB) and intensive care. An extensive series of experiments in dog, swine and rat models have demonstrated its efficacy and clinical feasibility.[2–12] The importance of optimizing EPR in experimental models has taken on greater significance given the recent funding of the first clinical feasibility trial of this strategy. In dogs, the rapid induction of profound cerebral hypothermia by aortic flush of cold saline to tympanic temperature (Tty) 10 °C, immediately after the start of exsanguination CA, can achieve survival without functional or histologic brain damage, after CA of up to 180 min.[5] Although good outcomes were achieved in the studies using a dog model, there are limitations to these large animal experiments including the need for long-term intensive care (72—96 h after resuscitation), lack of molecular tools to study mechanisms of brain injury, and cost. A rat model of EPR has been developed recently by our group.[12] In rats, EPR of 60 min CA is survivable, while 75 min CA is associated with substantial mortality and impaired neurological outcome in survivors.[13] In this report, we initiated studies to explore the possible molecular mechanisms at the threshold of failure of EPR. Two mechanisms of secondary injury that have been suggested to play important roles in cerebral ischemia are nitration and poly (ADP-ribose) polymerase (PARP) activation. Richards et al.[14] recently reported an important role for nitration of pyruvate dehydrogenase (PDH) and other targets in standard resuscitation after CA, while PARP activation has been shown to have a key role in limiting cerebral recovery across a variety of insults.[15,16] We, thus, hypothesized that nitration and PARP activation would be increased after 75 min CA treated by EPR compared to 60 min EPR or controls. We also explored whether CPB alone was associated with nitration or PARP activation.

Methods

The protocol for the study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Adult male Sprague-Dawley rats (350—400 g; Hilltop Lab Animals; Scottdale, PA) were housed in the facility for at least 3 days before the experiment with unrestricted access to food and water. Four groups were studied in this protocol: naïve, CPB controls, 60 min EPR and 75 min EPR.

Animal protocol

Rats were anesthetized with 4% isoflurane in FiO_2 1.0 for 5 min in a transparent plexiglas chamber, intubated with a 14G intravenous cannula (Becton Dickinson; Sandy, UT) and mechanically ventilated (Harvard Ventilator Model 683, Harvard Rodent Apparatus; South Natick, MA). Anesthesia was maintained with 1—2% isoflurane in FiO_2 0.5. Utilizing asepsis, the left femoral artery and vein were cannulated. EKG, respiration, arterial and central venous pressure were continuously monitored and recorded (Polygraph; Grass Instruments, Quincy, MA). The right femoral artery was cannulated with a 20G catheter (Becton Dickinson; Sandy, UT), that served as an arterial CPB cannula. The right jugular vein was cannulated with a modified five-hole 14G cannula advanced to the right atrium. This cannula was used for venous drainage during the hemorrhage phase and later as a venous CPB cannula. Rectal and tympanic probes were used to monitor the temperature. Heparin was administered to achieve activated clotting time (ACT) >400 s (Haemochron Jr. Signature, ITC; Edison, NJ). After instrumentation, rats were allowed to breathe spontaneously 2% isoflurane in FiO_2 0.25.

After a 5 min equilibration period, a rapid exsanguination (12.5 ml of blood over 5 min) was performed via the external jugular catheter and shed blood was collected for re-transfusion. CA was ensured with administration of a mixture of esmolol (9 mg) and potassium chloride (KCl, 0.2 mEq) intravenously after the exsanguination phase. At CA 1 min, the flush solution (0—2 °C Plasma-Lyte A, Baxter; Deerfield, IL) was administered retrogradely into the aorta via the right femoral artery catheter at 50 ml/min using a roller pump, and was drained from the jugular vein catheter. A target Tty of 15 °C during CA was achieved with a combination of 270 ml of flush and surface cooling started at CA 1 min. After 60 min (60 min EPR group) or 75 min (75 min EPR group) CA, resuscitation was started with CPB. CPB controls (without exsanguination, flush, or no-flow period) were also studied. Identical doses of KCl and esmolol were used to induce asystole, followed immediately by 60 min of CPB at 34 °C.

The CPB circuit consisted of a custom-designed oxygenator, an open reservoir (Ing. Martin Humbs, Ingenierburo fur Feinwerktechnik, Munich, Germany), tubing, and a roller pump (Masterflex, Barnant; Barrington, IL). The oxygenator contained a three-layer capillary membrane sufficient to provide oxygen levels >400 mm Hg. A mixture of $98\%O_2/2\%CO_2$ was used at a flow rate of 50 ml/min via the CPB circuit to provide oxygenation and prevent hypocapnia. Isoflurane from a separate vaporizer was used for maintenance of anesthesia during CPB. Heating and cooling were achieved with a circulating water bath and a forced-air blower (Life-Air 1000, Progressive Dynamics, Marshall, MI) blown over the rat covered with a semi-closed transparent