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EXPERIMENTAL PAPER

Assessment of the delta opioid agonist DADLE in a rat model of lethal hemorrhage treated by emergency preservation and resuscitation☆

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Summary Emergency preservation and resuscitation (EPR) is a new approach for resuscitation of exsanguination cardiac arrest (CA) victims. EPR uses a cold aortic flush to induce deep hypothermic preservation during no-flow to buy time for transport and damage control surgery, followed by resuscitation with cardiopulmonary bypass (CPB). We reported previously that 20—60 min EPR in rats was associated with intact outcome, while 75 min EPR resulted in high mortality and neurological impairment in survivors. The delta opioid agonist DADLE ([D-Ala(2),D-Leu(5)]-enkephalin) was shown previously to be protective against ischemia-reperfusion injury in multiple organs, including brain. We hypothesized that DADLE could augment neurological outcome after EPR in rats. After rapid lethal hemorrhage, EPR was initiated by perfusion with ice-cold crystalloid to induce hypothermia (15 °C). After 75 min EPR, resuscitation was attempted with CPB. After randomization, three groups were studied ($n = 10$ per group): DADLE 0 mg/kg (D0), 4 mg/kg (D4) or 10 mg/kg (D10) added to the flush and during reperfusion. Survival, overall performance category (OPC; 1 = normal, 5 = death), neurological deficit score (NDS; 0—10% normal, 100% = max deficit), and histological damage score (HDS) were assessed in survivors on day 3. In D0 group, 2/10 rats survived, while in D4 and D10 groups, 4/10 and 5/10 rats survived, respectively (p = NS). Survival time (h) was 26.7 ± 28.2 in D0, 36.3 ± 31.9 in D4 and 47.1 ± 30.3 in D10 groups, respectively ($p = 0.3$). OPC, NDS and HDS were not significantly

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different between groups. In conclusion, DADLE failed to confer benefit on functional or histological outcome in our model of prolonged rat EPR.

Introduction

Currently, the outcomes from traumatic exsanguination cardiac arrest (CA) are poor. Data from both recent military conflicts[1] and civilian settings[2,3] show that over 50% of deaths due to trauma occur at the scene, where medical care is limited. However, in an appropriate setting, some of those injuries could be surgically repairable.[4]

Emergency preservation and resuscitation (EPR) is a new approach for resuscitation of CA victims.[5] EPR uses cold aortic flush to induce hypothermic preservation during circulatory arrest and buys time for transport, damage control surgery, and delayed resuscitation with cardiopulmonary bypass (CPB). Neurological outcome after EPR is dependent on the duration of CA and temperature in both large (dog, swine) and small animal models. Given the logistic challenges of inducing hypothermia and potential risks of hypothermia, testing of potential pharmacological adjuncts that would allow extension of the period of CA is warranted.

The delta receptor agonist ([D-Ala(2),D-Leu(5)]-enkephalin—DADLE) has been recently evaluated as a possible link to hibernation.[6] DADLE possesses organ preservation properties evaluated on liver,[7] heart [8–12] and lungs,[13] or the organs harvested *en bloc*.[14] The vast majority of the experiments revealed positive results, extending ischemic time while preserving post-reperfusion organ function. Neuroprotective properties were also observed in multiple scenarios, including global brain ischemia[15] or pharmacologically induced brain injury.[16,17] Moreover, DADLE effects were exerted even under hypothermia.[8,13]

In our previous studies, we established a rat model of EPR with excellent survival and neurological recovery after 20—60 min CA.[18] Further extension of the EPR duration to 75 min resulted in high mortality resulting from multi-organ failure, and neurological impairment in survivors.[19]

Using this model, we hypothesized that DADLE would augment survival and neurological outcome after exsanguination CA followed by 75 min EPR. To test this hypothesis, we evaluated two doses of DADLE added to the flush and administered during reperfusion. We assessed survival rate, survival time and neurological outcome as primary outcome measurements. Histological damage score (HDS) served as a secondary outcome measurement.

Materials and methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Adult male Sprague—Dawley rats (350—400 g) were obtained from Hilltop Lab Animals (Scottdale, PA) and housed for at least 3 days before the experiment under 12-h light/dark cycle with unrestricted access to food and water. On the day of the experiment, rats were anesthetized with 4% isoflurane in a transparent acrylic jar. After tracheal intubation with a 14G intravenous catheter (Becton Dickinson; Sandy, UT), rats were mechanically ventilated using a piston ventilator (Harvard Ventilator Model 683, Harvard Rodent Apparatus; South Natick, MA) with a tidal volume of 0.8 ml/100 g and a frequency of 20—24 min^{-1} to maintain normocapnia, and a positive end-expiratory pressure (PEEP) of 4 cm H_2O. Anesthesia was maintained with 1.5—2% isoflurane in FiO_2 0.5. After shaving and cleansing with povidone iodine, bilateral femoral and right jugular cutdowns were performed. The left femoral artery and vein were cannulated for blood pressure monitoring and blood sampling. ECG, respiration, arterial and central venous pressure were monitored and recorded continuously (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 intravenous cannula advanced to the right atrium to be 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. After instrumentation, rats were weaned from the ventilator and allowed to spontaneously breathe 2% isoflurane in FiO_2 0.25 via a nose cone mask over the tracheal tube. Baseline blood samples were obtained, and hemodynamic values were recorded. Removed blood volume was replaced with an electrolyte-balanced crystalloid Plasma-Lyte A (Baxter; Deerfield, IL) in a ratio 1:3. Heparin sodium was given to achieve activated clotting time (ACT) >400 s (Haemochron Jr. Signature, ITC; Edison, NJ).

After a 5 min equilibration period, rapid exsanguination (12.5 ml of blood over 5 min) was performed via the internal jugular catheter. The shed blood was collected in a heparin pre-filled syringe. After the rapid exsanguination phase, CA was ensured with a mixture of 9 mg of esmolol and 0.1 ml of potassium chloride (0.2 mequiv.) intravenously. After 1 min of no-flow, 270 ml of an ice-cold flush solution (Plasma-Lyte A) was instilled via the right femoral artery catheter at 50 ml/min using a roller pump (Masterflex, Barnant, IL). The flush was drained from the jugular vein catheter. A target rectal temperature of 15 °C was achieved with a combination of 270 ml of flush and surface cooling. Both rectal and tympanic temperatures were maintained at 15 °C during EPR.

The rats were randomized into three groups ($n = 10$ per group): DADLE 0 mg/kg (D0), 4 mg/kg (D4) or 10 mg/kg (D10) added to the flush and during reperfusion. Rats in the D0 group received the same volume of vehicle (Plasma-lyte A) as rats in the D4 and D10 groups.

After 75 min of CA, resuscitation was started with CPB. (Fig. 1) In brief, the CPB circuit consisted of a custom-designed oxygenator made of polymethylmethacrylate, a reservoir (both made by Ing. Martin Humbs, Ingenierburo fur Feinwerktechnik, Munich, Germany), platinum-cured