Background—Induction of profound hypothermia for emergency preservation and resuscitation (EPR) of trauma victims who experience exsanguination cardiac arrest may allow survival from otherwise-lethal injuries. Previously, we achieved intact survival of dogs from 2 hours of EPR after rapid hemorrhage. We tested the hypothesis that EPR would achieve good outcome if prolonged hemorrhage preceded cardiac arrest.

Methods and Results—Two minutes after cardiac arrest from prolonged hemorrhage and splenic transection, dogs were randomized into 3 groups (n=7 each): (1) the cardiopulmonary resuscitation (CPR) group, resuscitated with conventional CPR, and the (2) EPR-I and (3) EPR-II groups, both of which received 20 L of a 2°C saline aortic flush to achieve a brain temperature of 10°C to 15°C. CPR or EPR lasted 60 minutes and was followed in all groups by a 2-hour resuscitation by cardiopulmonary bypass. Splenectomy was then performed. The CPR dogs were maintained at 38.0°C. In the EPR groups, mild hypothermia (34°C) was maintained for either 12 (EPR-I) or 36 (EPR-II) hours. Function and brain histology were evaluated 60 hours after rewarming in all dogs. Cardiac arrest occurred after 124±16 minutes of hemorrhage. In the CPR group, spontaneous circulation could not be restored without cardiopulmonary bypass; none survived. Twelve of 14 EPR dogs survived. Compared with the EPR-I group, the EPR-II group had better overall performance, final neurological deficit scores, and histological damage scores.

Conclusions—EPR is superior to conventional CPR in facilitating normal recovery after cardiac arrest from trauma and prolonged hemorrhage. Prolonged mild hypothermia after EPR was critical for achieving intact neurological outcomes. (Circulation. 2006;113:1974-1982.)

Key Words: cardiopulmonary bypass ■ cardiopulmonary resuscitation ■ heart arrest ■ hemorrhage ■ hypothermia

Clinical Perspective p 1982

Conventional resuscitation, including open cardiac massage, is often unsuccessful after exsanguination cardiac arrest in trauma victims, particularly when it results from prolonged hemorrhagic shock. A novel approach is needed. Previously, we reported the success of inducing emergency preservation and resuscitation (EPR) with profound hypothermia in animal models. The goal of EPR is to “buy time” for transport and resuscitative surgery during pulselessness, followed by delayed resuscitation. EPR of up to 2 hours was induced with a rapid aortic flush with ice-cold (2°C) saline to induce profound hypothermia, followed by delayed resuscitation with cardiopulmonary bypass (CPB). We used EPR to achieve intact survival of dogs after rapid hemorrhage (over 5 minutes) and cardiac arrest. The success of EPR relies on the timely initiation of preservation during cardiac arrest. When 30-minute EPR was delayed for 2 or 5 minutes after cardiac arrest, all dogs survived with good neurological function. However, when EPR was delayed by 8 minutes, none had good outcomes. We speculated that prolonged hemorrhagic shock before cardiac arrest may decrease the efficacy of EPR. Longer durations of hemorrhagic shock may cause severe tissue acidosis and exhaust reserves. Although the central nervous system may be damaged only minimally during hemorrhagic shock, superimposing transient, normothermic cardiac arrest and a period of EPR on prolonged hemorrhagic shock may substantially complicate efforts to save trauma victims.

In this study, we designed a model relevant to military and civilian trauma, characterized by rate-controlled bleeding, trauma (laparotomy and spleen transection), limited (hypoten-
Ketamine 10 mg/kg and atropine 0.4 mg were administered intra-
venously. After anesthesia induction with 4% halothane by face
mask, endotracheal intubation (internal diameter, 8 to 9 mm) was
performed. Continuous anesthesia was provided with ~1% hal-
othane, titrated during preparation with O2:N2O, 50%:50%. Controlled
ventilation (Piston Ventilator Model 613, Harvard Apparatus, South
Natwick, Mass) was initiated with a tidal volume of 12 to 15 mL/kg,
a positive end-expiratory pressure of 2 cm H2O, and a frequency of
20 to 25/min, titrated to maintain a Paco2 of 35 to 45 torr. ECG lead
II was continually monitored. A cannula (18 gauge) was inserted into
a peripheral vein, and fluid infusion (D,W/0.45% NaCl at 4 mL ·
kg⁻¹ · h⁻¹) was started. A Foley catheter was placed. Sterile cutdowns
were performed in both groins and the right side of the
neck. Temperature probes were inserted for measuring rectal, esopha-
geal, and both tympanic membrane temperatures (Tty). A PE-90
catheter was inserted into the left femoral arterial for blood pressure monitoring and blood sampling. A pulmonary artery catheter (7.5F)
was inserted via the left femoral vein to monitor pressure, cardiac output, and core temperature (Tpa). A CPB arterial cannula (7 or 9
gauge) was inserted into the right femoral arterial. A multiple-hole
cannula (16F) was inserted into the inferior vena cava via the right
femoral vein for blood withdrawal. Another multiple-hole cannula
(19F) was inserted 10 cm into the right external jugular vein. This
cannula was advanced into the right atrium when mean arterial
pressure (MAP) was 30 mm Hg during hemorrhagic shock. The
cannulas were flushed intermittently with diltiazem and heparinized saline.

The CPB system, including an oxygenator (Medtronic, Grand
Rapids, Mich) and centrifugal pump (Biomedicus, Eden Prairie,
Minn), was primed with shed blood (30 mL/kg) and Plasma-Lyte A
(Baxter, Deerfield, Ill). In the CPR group, 500 U of heparin was
administered during resuscitation. Temperature and MAP values are shown.

Support guidelines plus aggressive fluid resuscitation; (2) the EPR-I
and EPR-II groups were resuscitated with conventional Advanced
Cardiac Life Support protocols. In brief, chest compressions with a mechanical thumper (Michigan Instruments, Grand
Rapids, Mich) were initiated at 60/min; the compressing distance was
adjusted to generate a systolic blood pressure of 100 mm Hg.

Hemorrhagic Shock and Cardiac Arrest Phase
All heating sources were stopped. At hemorrhagic shock time 0
minutes, continuous venous blood withdrawal via the right femoral
vein catheter was set at 1 mL · kg⁻¹ · min⁻¹ over 40 minutes. Withdrawn blood was anticoagulated with 0.125 mL · kg⁻¹ · min⁻¹
saline, followed by infusion of shed blood (30 mL/kg) over 5 minutes. Up

Hemorrhagic Shock and Cardiac Arrest Phase
All heating sources were stopped. At hemorrhagic shock time 0
minutes, continuous venous blood withdrawal via the right femoral
vein catheter was set at 1 mL · kg⁻¹ · min⁻¹ over 40 minutes. Withdrawn blood was anticoagulated with 0.125 mL · kg⁻¹ · min⁻¹
citrate delivered through a PE-60 catheter in the femoral vein
to simulate spontaneous breathing. At hemorrhagic shock time 40 minutes, the spleen was transected, and the blood withdrawal rate was
decreased to 0.5 mL · kg⁻¹ · min⁻¹. Halothane was decreased to 0.5%
when MAP was <50 mm Hg. When MAP reached <30 mm Hg, limited fluid resuscitation (simulating field resuscitation) was started
with bolus infusions of lactated Ringer’s solution (100 mL over 2
minutes), with a maximum volume of 500 mL. Cardiac arrest was
defined as either an MAP <10 mm Hg and severe bradycardia (<20
bpm) or asystole or ventricular fibrillation.

CPR/EPR Phase
Two minutes after cardiac arrest, dogs were randomized into the
CPR or EPR groups. In the CPR group, conventional Advanced
Cardiac Life Support protocols were initiated. In brief, chest com-
pressions with a mechanical thumper (Michigan Instruments, Grand
Rapids, Mich) were started at 60/min; the compressing distance was
adjusted to generate a systolic blood pressure of 100 mm Hg.
to 3 additional boluses of lactated Ringer’s solution (250 mL over 15 minutes) were administered per Advanced Trauma Life Support recommendations.

In the EPR groups, the lungs were inflated with air to maintain an airway pressure of ~10 cm H₂O during EPR. An aortic flush of 20 L of 2°C saline via the right femoral arterial cannula was initiated at 1.6 L/min with use of a roller pump (Ardiem, Indiana, Pa). The flush solution was drained through the external jugular catheter. The dog was then covered with ice.

Delayed Resuscitation Phase (RT 0 to 2 Hours)
Sixty minutes after the onset of aortic flush or CPR, CPB was started. Just before CPB, additional heparin (1500 U) and sodium bicarbonate (2 mEq/kg) were injected into the circuit. Dogs were paralyzed with pancuronium. CPB was started at 100 mL·kg⁻¹·min⁻¹. Reinfusion of shed blood in the EPR groups was titrated to achieve a central venous pressure of 10 to 15 mm Hg. Repetitive doses of epinephrine (0.01 mg/kg) were given when necessary to achieve a central venous pressure of 10 to 15 mm Hg. O₂ flow through the CPB oxygenator was adjusted to keep the PaCO₂ at 30 to 35 mm Hg. Ventilation at a rate of 8 to 10/min was resumed to prevent atelectasis. IV fluids were restarted at 100 mL/h. A base deficit of >6.0 mEq/L was corrected with sodium bicarbonate. CPB flow was reduced to 75 mL·kg⁻¹·min⁻¹ at 60 minutes and to 50 mL·kg⁻¹·min⁻¹ at 90 minutes. During CPB, activated clotting times were maintained at >300 seconds with heparin.

At RT 0 minutes, a splenectomy was performed, and the abdomen was packed with gauze to simulate the clinical management of a trauma victim with a ruptured spleen. An abdominal drainage catheter was placed through the abdominal wall. The abdominal wound was closed. Tpa in the CPR group was maintained at 1.6 L/min with use of a roller pump (Ardiem, Indiana, Pa). The flush solution was drained through the external jugular catheter. The dog then covered with ice.

Intensive Care Management (RT 2 to 24 Hours in the CPR and EPR-I Group or 48 Hours in the EPR-II Group)
Neuromuscular blockade was maintained with intermittent doses of pancuronium (0.1 mg/kg). Sedation and analgesia was provided with N₂O/O₂ (50%:50%) plus IV boluses of morphine (0.1 to 0.3 mg/kg) and diazepam (0.1 to 0.2 mg/kg) to prevent signs of wakefulness, eg, mydriasis. Severe hypertension (MAP > 150 mm Hg) despite adequate analgesia was controlled with IV boluses of labetalol (0.25 to 0.5 mg/kg) or hydralazine (0.1 to 0.2 mg/kg). Hypotension (MAP < 70 mm Hg) was treated by normalization of filling pressures by administration of lactated Ringer’s solution and titrated norepinephrine. The dogs received ceftazolin (250 mg IV) every 8 hours for infection prophylaxis.

Intensive care unit (ICU) care, including mechanical ventilation, was provided for at least 24 hours in the CPR and EPR-I groups and for 48 hours in the EPR-II group to ensure an equivalent period of postresuscitative care. At 20 hours, the abdominal packing was removed and the abdominal wall closed. In the CPR group, body temperature was maintained at 37.5°C to 38.5°C throughout the experiment. In the EPR-I group, body temperature was maintained at 34°C until RT 12 hours, which was followed by self-rewarming and, when needed, external heating with blankets and a heater (target rewarming rate, 1°C/h) to 37.5°C. In the EPR-II group, rewarming was delayed to 36 hours and was deliberately slower (0.3°C/h).

Outcome Evaluation
Functional outcomes were evaluated after discontinuing sedation according to overall performance categories (1 = normal [able to eat and walk]; 2 = moderate disability [able to eat and sit but not stand]; 3 = severe disability [responseto pain but unaware of the environment]; 4 = coma [minimal response to pain; positive pupillary light reflex; running movements and opisthotonus common]; and 5 = death) and neurological deficit scores. The neurological deficit score is based on assessment of 5 facets of neurological function (level of consciousness, breathing pattern, cranial nerve function, sensory and motor function, and behavior), each with a maximum value of 20% (neurologic deficit score 0% to 10% = normal; 100% = brain death). Evaluations were agreed on by at least 2 team members. Because of the number of team members needed to conduct these experiments and the differences in observation time between groups, there was no practical way for the evaluations to be blinded. In previous experiments, interobserver agreement has been excellent. Results at 60 hours after initiation of rewarming were taken as the final measurements in each group. Blood samples were obtained at baseline and every 24 hours for cardiac (troponin I, creatine phosphokinase MB fraction), and liver (transaminases and bilirubin) enzymes. At 72 hours (EPR-I) or 96 hours (EPR-II), animals were reanesthetized with ketamine and halothane. A left thoracotomy was performed. Perfusion-fixation of the brain was accomplished with aortic infusion of 4% paraformaldehyde. A gross necropsy was performed. The brain was removed ≈1 to 2 hours after perfusion-fixation and retained in 10% neutral buffered formalin until dissection.

Neuropathology
Whole perfusion-fixed brains were divided into multiple coronal slices. Six coronal brain slices plus 3 transverse sections of the medulla oblongata and upper cervical cord were selected for microscopic evaluation. These slices were taken at the following levels: (1) optic chiasm; (2) anterior thalamus; (3) midbrain; (4) posterior portions of the occipital lobes; (5) midbrain and underlying brain stem; and (7) medulla oblongata and upper cervical cord. Brain slices were processed for paraffin embedding, resulting in 2 tissue blocks per brain. Blocks were sectioned at 5 μm, and the sections were stained with hematoxylin/eosin and Fluoro-Jade B. The examining neuropathologist (R.G.) was blinded to treatment. A total of 25 neuroanatomic regions were examined. Each region with damage on microscopic examination received a pathological grade ranging from 1+ (minimal) to 5+ (severe). Each affected region on each side of the brain received separate scores in hematoxylin/eosin-stained and Fluoro-Jade B-stained sections. In each region, scores for edema were multiplied by 1, and scores for neuronal degeneration were multiplied by 2. Edema was not scored on the Fluoro-Jade B sections. Thus, the total possible scores for each region were 5 × 1 plus 5 × 2 (total, 15) for the hematoxylin/eosin stain and 5 × 2 (total, 10) for the Fluoro-Jade B stain. Total histological damage scores were determined by totaling these individual scores (ie, for each region with each stain). The maximum score was 1250 ((15+10 maximum per region)×25 regions×2 sides of the brain).

Statistical Analysis
Data are presented as mean ± SD unless otherwise stated. A repeated-measures ANOVA was performed, followed by Bonferroni post hoc tests to identify differences in hemodynamic parameters, temperature, and neurological deficit and histological damage scores (with ranked data). ANOVA was performed for other physiological variables. The Mann-Whitney U test was used for the final neurological deficit score and the total histological damage score. The Fisher exact test was used to assess differences in overall performance category proportions (ie, normal outcome [overall performance category 1] versus abnormal outcome) among groups. A probability value < 0.05 was considered significant.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Baseline
Baseline hemodynamics, hematological and biochemical parameters, and acid-base status were similar among groups.
Hemorrhagic Shock and Cardiac Arrest
The hemorrhage time before cardiac arrest was 124 ± 16 minutes and did not differ among groups (Table 1). Samples of arterial blood gases and chemistries taken 1 minute after cardiac arrest were markedly abnormal but did not differ among groups (Table 1).

Resuscitation of the CPR Group
During chest compressions, MAP was maintained at >50 to 60 mm Hg (Figure 2). However, return of spontaneous circulation (ROSC) was not achieved by CPR in any dog.

During CPB, ROSC was achieved with defibrillation (mean total defibrillation energy, 157 ± 181 J) in all dogs 15 ± 16 minutes after initiation of CPB. However, substantial fluid losses from the rectum, orogastric tube, and intraperitoneal drain occurred during the resuscitation phase (Table 2; all P < 0.01 versus the EPR groups). Progressive hypotension developed despite massive fluid resuscitation and vasoactive support. Lactate levels decreased transiently but increased sharply again until death (Figure 3; *P < 0.01 versus the EPR groups). Tty decreased slightly during CPR to ~36°C and then increased to 38°C with CPB (Figure 4).

Resuscitation of EPR Groups
The perfusion pressure during aortic flush was ~20 mm Hg, with no difference between the EPR groups (Figure 2). At the end of the aortic flush, Tty had decreased to similar levels, with little change during circulatory arrest (Figure 4).

EPR dogs were rewarmed to 34°C within 1 hour by CPB. When Tpa reached 32°C, defibrillation yielded ROSC in all dogs at 30 ± 12 minutes in the EPR-I group and at 32 ± 23 minutes in the EPR-II group (P = NS). The total defibrillation energy required was 229 ± 225 J in the EPR-I group and 264 ± 326 J in the EPR-II group (P = NS).

At RT 12 hours, hematocrit was lower in the CPR group (19 ± 9%) compared with the EPR-I (33 ± 7%, P = 0.006) and EPR-II (28 ± 9%, P = 0.16) groups. Final hematocrit values were 29 ± 3% at RT 24 hours in the EPR-I group and 30 ± 6% at RT 48 hours in the EPR-II group (P = NS versus the EPR-I group).

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**TABLE 1. Physiological Parameters During Cardiac Arrest After Prolonged Hemorrhage**

<table>
<thead>
<tr>
<th></th>
<th>CPR Group</th>
<th>EPR-I Group</th>
<th>EPR-II Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhage time, min</td>
<td>124.4 ± 10.5</td>
<td>118.4 ± 19.7</td>
<td>126.4 ± 19.8</td>
</tr>
<tr>
<td>pH</td>
<td>6.88 ± 0.24</td>
<td>6.99 ± 0.16</td>
<td>6.93 ± 0.12</td>
</tr>
<tr>
<td>Pco₂, mm Hg</td>
<td>86 ± 39</td>
<td>58 ± 30</td>
<td>73 ± 28</td>
</tr>
<tr>
<td>Po₂, mm Hg</td>
<td>58 ± 8</td>
<td>85 ± 31</td>
<td>79 ± 24</td>
</tr>
<tr>
<td>Base deficit, mmol/L</td>
<td>16.6 ± 2</td>
<td>16.4 ± 1.3</td>
<td>15.5 ± 1.8</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>7.6 ± 1.2</td>
<td>6.8 ± 1.3</td>
<td>7.3 ± 0.9</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>449 ± 150</td>
<td>563 ± 110</td>
<td>522 ± 207</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>15.1 ± 1.6</td>
<td>14.6 ± 2.8</td>
<td>14.1 ± 2.3</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL</td>
<td>23.4 ± 6.1</td>
<td>27.6 ± 6.5</td>
<td>25.8 ± 8.1</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>16.2 ± 2.2</td>
<td>18.1 ± 2.3</td>
<td>19.6 ± 1.8</td>
</tr>
</tbody>
</table>

**TABLE 2. Fluid Balance During the First 16 Hours of Resuscitation**

<table>
<thead>
<tr>
<th></th>
<th>CPR Group</th>
<th>EPR-I Group</th>
<th>EPR-II Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid requirement, L</td>
<td>15.3 ± 2.6*</td>
<td>4.1 ± 0.6</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>Gastrointestinal fluid loss, L†</td>
<td>5.1 ± 1.7*</td>
<td>0.7 ± 0.4</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Urine output, L</td>
<td>0.03 ± 0.08*</td>
<td>2.0 ± 1.2</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>Abdominal drainage, L</td>
<td>2.4 ± 1.2*</td>
<td>0.3 ± 0.2</td>
<td>0.5 ± 0.4</td>
</tr>
</tbody>
</table>

*P < 0.01, compared with EPR groups.
†Includes orogastric and rectal fluid losses.

---

Figure 2. MAP during CPR or EPR, followed by delayed resuscitation. *P < 0.01, comparing the CPR group with either EPR group.
Final Outcome of All Groups

All CPR dogs died, with a median survival time of 14.7 hours (range, 11.5 to 16.5 hours; \( P < 0.01 \) versus the EPR groups). In contrast, 6 of 7 EPR-I dogs survived to 72 hours; 1 died at RT 29.5 hours due to circulatory collapse. Similarly, in the EPR-II group, 6 of 7 dogs survived to 96 hours; 1 dog unexpectedly died at RT 66 hours. This dog was extubated at 48 hours and was in overall performance category 1. At necropsy, gastric contents were found inside the trachea and bronchus, suggesting aspiration.

Thirty-six hours after rewarming (RT 24 hours in the EPR-I group or RT 48 hours in the EPR-II group), 3 of 6 surviving dogs in the EPR-I group and 4 of 6 surviving dogs in the EPR-II group were in overall performance category 1 or 2. In the EPR-I group, however, 2 dogs developed recurrent generalized seizures 24 hours after extubation. Another had seizures shortly after extubation at RT 24 hours. Neurological function deteriorated in these animals. There was only 1 dog that recovered to overall performance category 1. In contrast, none of the EPR-II survivors exhibited seizures, and 5 of 6 survivors continued to improve and regained normal function (\( P = 0.06 \) versus the EPR-I group; Figure 5). The neurological deficit score of the survivors in the EPR-II group was numerically better over time compared...
with the EPR-I group ($P=0.09$), although the final neurological deficit scores were better ($P=0.04$; Figure 6).

### Brain Histology

In the EPR-I group, the brains exhibited prominent, acute eosinophilic neuronal degeneration within the frontal, parietal, temporal, and occipital cortices (Figure 7). In the most severely affected regions, a laminar band of necrosis with associated neuropil spongiosis was evident. Marked to severe neuronal degeneration was present in the caudate region, with slightly less degeneration in the putamen. Within the caudate, degeneration was primarily present in the medium spiny neurons. The larger interneurons were relatively unscathed. In the hippocampus, moderate to marked neuronal degeneration was present primarily within the CA1 region, although degeneration often extended to the CA3 and CA4 regions. In the cerebellum of the EPR-I group, Fluoro-Jade B staining revealed small numbers of degenerating Purkinje neuron cell bodies and greater numbers of dendrites. Degrees of neuronal degeneration were scored slightly higher in Fluoro-Jade B–stained sections than in hematoxylin/eosin-stained sections.

In contrast to the EPR-I group, the EPR-II group had only mild damage in the neocortex (Figure 7A; $P<0.05$ versus EPR-I in the frontal and temporal lobes). In the hippocampus of the EPR-II group, damage severity as assessed by Fluoro-Jade B staining was also less (Figure 7B; $P=0.065$). Furthermore, Fluoro-Jade B staining in the hippocampus of EPR-II group dogs was primarily restricted to neuronal processes within CA3 and CA4. Degenerative changes in the caudate and putamen of EPR-I and EPR-II groups were similar (Figure 7). Within the cerebellum of the EPR-II group, Fluoro-Jade B staining revealed scattered Purkinje neurons with degenerative dendrites.

Figure 7 includes only those brain regions that had greater than minimal degrees of degenerative changes. All other brain regions, ie, the piriform cortex, entorhinal cortex, septal region, basal forebrain, anterior thalamus, posterior thalamus, amygdala, midbrain, pons, and medulla oblongata, were characterized by no or only minimal degrees of damage, with no differences between groups. By repeated-measures ANOVA for brain regions, the histological damage scores were higher in the EPR-I group than in the EPR-II group ($P=0.006$). The total histological damage scores in the EPR-I group (226±111) were numerically higher than those in EPR-II (102±87; $P=0.15$).

### Extracerebral Organ Injury

The heart and liver enzyme levels in the EPR groups were markedly increased after resuscitation. They decreased gradually after 24 hours but remained increased at 72 or 96 hours, with no difference between groups (data available in Data Supplement Table I).

At necropsy, in the CPR group, all dogs had generalized edema, severe endocardial hemorrhage, lung edema, and bloody ascites. Although the serosal side of the intestine had mild hemorrhage, the intestinal mucosa was sloughed over the entire length of the intestine. In the EPR groups, the most consistent findings were mild to moderate endocardial hemorrhage and extensive hemorrhage in the gallbladder wall. Occasionally, hemorrhagic spots were found on the serosal surface of the intestine, but there was no necrosis. Lung edema was found only in 1 EPR-I dog.

### Discussion

We established an exsanguination cardiac arrest model that is unsalvageable by contemporary conventional resuscitation. At the time of cardiac arrest, 60% to 90% of the estimated blood volume was removed. Arterial blood gases taken at the beginning of cardiac arrest revealed severe acidemia (pH<7.0), hyperkalemia, and hyperlactemia. As expected, none of the dogs could be resuscitated with CPR, despite vigorous blood and fluid replacement, standard drug therapy, and chest compressions with an MAP at 50 to 60 mm Hg. Such favorable hemodynamic responses would be difficult to achieve in traumatic, exsanguination cardiac arrest victims. Although all of the dogs could be resuscitated with CPB, all subsequently died of severe multiple-organ failure, including cardiovascular dysfunction, renal failure, and extensive gastrointestinal mucosal necrosis. This pattern is anticipated after prolonged hemorrhagic shock and cardiac arrest. Such trauma victims similarly develop irreversible shock, including vasodilatation unresponsive to vasopressors and massive capillary leak, presumably secondary to the systemic inflammatory response. Thus, it was anticipated that animals in the
conventional-resuscitation arm would exhibit considerable gastrointestinal fluid loss and ascites. We suspect that the large amounts of fluids administered in an attempt to sustain these animals led to hemodilution, as reflected in lower hematocrit values at 12 hours; there were no intergroup differences in blood loss. In contrast, EPR with delayed resuscitation was superior to conventional CPR for resuscitation of traumatic, exsanguination cardiac arrest.1–3 Twelve of 14 dogs survived without severe extracerebral organ damage.

Capone et al17 also compared EPR with conventional resuscitation. That investigation differed from the current study in that before

![Figure 7](https://example.com/fig7.png)

**Figure 7.** Brain histological damage scores (refer to text for details) after prolonged hemorrhagic shock leading to cardiac arrest with resuscitation by EPR, with or without prolonged postresuscitation mild hypothermia. Brain regions with no or minimal neuronal changes are not included. A, Hematoxylin/eosin staining; B, Fluoro-Jade B staining. Bars represent medians and interquartile ranges. Cx indicates cortex; N, neurons. *P < 0.05 for comparison between EPR-I and EPR-II; †P = 0.09, ‡P = 0.07.
induction of EPR, the period of severe hemorrhage was shorter (60 minutes), and there was no period of normothermic cardiac arrest. After profound hemorrhagic shock (MAP of 30 mm Hg for 60 minutes), survival was similar after conventional resuscitation or after addition of 1 hour of EPR at 10°C. The addition of more prolonged hemorrhagic shock and brief cardiac arrest before standard resuscitation in the current study is 100% lethal with standard care, although EPR can yield normal recovery.

Dogs in the EPR-I group had severe neurological deterioration after an initial recovery. Three dogs had generalized seizures, although 2 had regained consciousness initially. This is a unique pattern that we had not previously encountered during years of experience with exsanguination and normovolemic cardiac arrest models. Extensive neocortical laminar necrosis was found, along with cerebellar synaptic injury. This is in sharp contrast to the lack of histological brain damage after rapid exsanguination cardiac arrest in our prior EPR report.28 Given that the EPR and delayed resuscitation protocols were almost identical between these 2 studies, the preexisting prolonged hemorrhagic shock probably set the stage for this delayed neurological deterioration.

We speculate that the mechanism of this delayed neurological deterioration may be cytotoxic brain edema that peaks at \( \approx 48 \) hours after reperfusion.19 Different from rapid exsanguination cardiac arrest, blood glucose levels at the time of cardiac arrest in this model were \( >500 \) mg/dL in most dogs, likely related to the stress of prolonged hemorrhagic shock. On the basis of a histological pattern similar to that found after hyperglycemia-associated brain injury in forebrain ischemia models20 and previous reports that hyperglycemia exacerbates neurological dysfunction after cardiac arrest,21 we speculate that hyperglycemia may have contributed to the delayed neurological deterioration. Hypothermia appears to be very effective in protecting against ischemic brain injury during hyperglycemia.22 However, rapid rewarming of the traumatically injured brain can markedly exacerbate injury.23 This suggests the need to carefully optimize the use of mild hypothermia and rewarming.

The effects of prolonged post-EPR mild hypothermia were impressive. Similar to our findings, Gunn et al19 documented that delayed brain edema that peaked 48 hours after 30 minutes of cerebral ischemia in fetal lambs was abolished by prolonged (72-hour) hypothermia; 48-hour cooling was associated with rebound seizures. Likewise, Colbourne and Corbett24 found that to salvage CA1 neurons after 5 minutes of ischemia in gerbils, hypothermia (32°C), induced 1 hour after ischemia, had to be maintained for 24 hours. In our study, with hypothermia for 36 hours and slow rewarming, 5 of 7 dogs regained consciousness, and seizures were not observed. One dog died of probable aspiration. Although mild hypothermia is recommended for comatose survivors of cardiac arrest25 and laboratory studies suggest the benefit of mild hypothermia during resuscitation from hemorrhagic shock,26 more research on the optimal timing and rate of rewarming is needed.

This study has some limitations. First, it was not fully randomized. Seven EPR-II dogs were added after 5 experiments had been performed in each of the other groups because we had observed delayed neurological deterioration and we hypothesized that prolonged mild hypothermia with slow rewarming would prevent this deterioration. However, these studies were carried out in sequence, contiguous with those in the previous dogs by the same experienced research team. In addition, outcomes in the CPR and EPR-I experiments performed after the addition of the EPR-II group mirrored the earlier experiments.

Second, final outcome was determined at different times in the EPR-I and EPR-II groups. On the basis of our previous experience,4–10 we thought it essential to compare functional outcomes after identical postrewarming and extubation periods in the EPR-I and EPR-II groups. This mandated that we compare outcomes at 72 and 96 hours in the EPR-I and EPR-II groups, respectively. We recognize that for histological evaluations, delayed neuronal death may occur 3 days to 1 week after global ischemia,27 which may have biased the outcome toward worse damage at 96 hours than at 72 hours in the EPR-II group. Despite these differences, we still found significantly better brain histology results in the EPR-II group.

Third, external chest compressions are not standard care for trauma victims who experience cardiac arrest. Emergency department thoracotomy with open chest cardiac massage is indicated because of the potential for treating a surgical cause of cardiac arrest, eg, pericardial tamponade.3 External chest compressions generated excellent blood pressures with aggressive fluid resuscitation. Despite this, there were no long-term survivors in the CPR group.

Fourth, systemic heparinization was used, because standard CPB equipment was utilized. This precluded studies of coagulation. In the massively traumatized patient, however, one could avoid systemic heparin by using a heparin-bonded CPB system.

Fifth, vessel cannulation for EPR within 2 minutes of cardiac arrest may be difficult in the field, although it is reasonable for the scenario in the Emergency Department by a trauma surgeon. Also, large amounts of fluid are currently required to achieve the desired brain temperature. Despite these limitations, we believe that clinical application of EPR should be studied in trauma victims who have experienced exsanguination cardiac arrest. Ideally, this would be applied in the field. Ambulances could carry large amounts of fluid for the aortic flush. Given the complexity of the procedure with currently available equipment, however, we believe that the first study should be conducted in Emergency Departments of major trauma centers. We are working with companies to develop better vessel cannulation techniques, novel catheters, and cooling systems to facilitate the induction of EPR by civilian emergency medical services and perhaps, on the battlefield. This could also facilitate rapid initiation of CPB for delayed resuscitation in this situation, although this is less of an issue, because preparations for CPB can proceed simultaneously with resuscitative surgery.

EPR is remarkably superior to conventional CPR in facilitating survival and neurological recovery in a model of otherwise unrecoverable prolonged hemorrhage with exsanguination cardiac arrest. Extended application of mild hypothermia with slow rewarming during ICU care after EPR was critical in achieving intact neurological outcomes. Use of mild hypothermia and/or slow rewarming may also have implications for optimal neuroprotection in conventional deep hypothermic circulatory arrest.28

Acknowledgments

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Disclosures
Drs Wu, Kochanek, Tisherman and S.W. Stezoski have submitted a provisional patent entitled “Method of Inducing Suspended Animation Following Cardiopulmonary Arrest.” The other authors report no conflicts.

References

CLINICAL PERSPECTIVE
The success of emergency preservation and resuscitation (EPR) may have important implications. First, the potential for its use may not be limited by the duration of preexisting hemorrhagic shock. This is clinically important, because the exact duration of hemorrhage is usually unknown, and prolonged hemorrhagic shock before cardiac arrest is common in the rural or military trauma situation. Second, EPR is much more reliable in preserving tissue viability during cardiac arrest than is optimized cardiopulmonary resuscitation and may allow intact survival. Third, the impressive benefits of prolonged postischemic mild hypothermia and slow rewarming may have implications for neuroprotection after deep hypothermic circulatory arrest used in cardiac or neurological surgery. Brain damage found in deep hypothermic circulatory arrest is similar to that seen in our study. The development of EPR has focused on the exsanguinating trauma patient. Although most trauma patients are young and have normal coronary arteries, similar to the animals in this study, exsanguination can occur in older patients with coronary artery disease. The effects of inducing EPR in such patients are difficult to predict, although one should keep in mind that the results of current management strategies for trauma patients who experience cardiac arrest are dismal for all victims. Rapid cooling of the heart might help preserve cardiac function, just as brain cooling protects neurological function.
Induction of Profound Hypothermia for Emergency Preservation and Resuscitation Allows Intact Survival After Cardiac Arrest Resulting From Prolonged Lethal Hemorrhage and Trauma in Dogs
Xianren Wu, Tomas Drabek, Patrick M. Kochanek, Jeremy Henchir, S. William Stezoski, Jason Stezoski, Kristin Cochran, Robert Garman and Samuel A. Tisherman

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Table 3. Extracerebral organ damage in the emergency preservation and resuscitation (EPR) groups.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Baseline</th>
<th>RT 24 h</th>
<th>RT 48 h</th>
<th>RT 72/96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin I (ng/ml)</td>
<td>EPR–I</td>
<td>0.1 (0.1-0.6)</td>
<td>46.8 (21.5-214.4)</td>
<td>18.2 (5.4-120.1)</td>
<td>16.3 (8.3-44.2)</td>
</tr>
<tr>
<td></td>
<td>EPR–II</td>
<td>0.1 (0.1-0.1)</td>
<td>37.8 (7.3-60.1)</td>
<td>33.7 (12.7-76.1)</td>
<td>11 (5-65.5)</td>
</tr>
<tr>
<td>Total CPK (x10^3IU/L)</td>
<td>EPR–I</td>
<td>0.4 (0.4-0.6)</td>
<td>59.9 (48.8-134.2)</td>
<td>105.5 (18.6-182.1)</td>
<td>30.7 (14.4-84.1)</td>
</tr>
<tr>
<td></td>
<td>EPR–II</td>
<td>0.3 (0.3-0.5)</td>
<td>96.3 (29.4-210.5)</td>
<td>48.1 (38.6-118.2)</td>
<td>16.7 (6.4-102.8)</td>
</tr>
<tr>
<td>CPK-MB (IU/L)</td>
<td>EPR–I</td>
<td>1.4 (0.3-2.8)</td>
<td>31.6 (25.3-53.4)</td>
<td>12.6 (10.6-18)</td>
<td>5.3 (4.8-9.7)</td>
</tr>
<tr>
<td></td>
<td>EPR–II</td>
<td>1 (0.7-2.1)</td>
<td>61.0 (32.7-120)</td>
<td>43.0 (27.3-125.5)</td>
<td>5.2 (1.9-45)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>EPR–I</td>
<td>33 (27-40)</td>
<td>2480 (717-3587)</td>
<td>1318 (523-2493)</td>
<td>802 (517-1137)</td>
</tr>
<tr>
<td></td>
<td>EPR–II</td>
<td>32 (22-42)</td>
<td>1273 (556-2027)</td>
<td>887 (457-3260)</td>
<td>495 (209-2560)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>EPR–I</td>
<td>27 (24-51)</td>
<td>702 (211-3340)</td>
<td>617.5 (318-960)</td>
<td>634 (285-2525)</td>
</tr>
<tr>
<td></td>
<td>EPR–II</td>
<td>47 (22-73)</td>
<td>772 (512-1112)</td>
<td>781 (640-944)</td>
<td>901 (624-1414)</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>EPR–I</td>
<td>all&lt;0.1</td>
<td>all&lt;0.1</td>
<td>all&lt;0.1</td>
<td>all&lt;0.9</td>
</tr>
<tr>
<td></td>
<td>EPR–II</td>
<td>all&lt;0.1</td>
<td>all&lt;0.3</td>
<td>all&lt;0.1</td>
<td>all&lt;0.1</td>
</tr>
</tbody>
</table>

RT = resuscitation time; CPK = creatine phosphokinase; AST = aspartate aminotransaminase; ALT = alanine aminotransferase. Median (interquartile range). No differences between groups.