Intra-Arrest Cooling Improves Outcomes in a Murine Cardiac Arrest Model

Benjamin S. Abella, MD, MPhil; Danhong Zhao, MD, PhD; Jason Alvarado, BA; Kim Hamann, PhD; Terry L. Vanden Hoek, MD; Lance B. Becker, MD

Background—Recent clinical studies have demonstrated that hypothermia to 32°C to 34°C provides significant clinical benefit when induced after resuscitation from cardiac arrest. However, cooling during the postresuscitation period was slow, requiring 4 to 8 hours to achieve target temperatures after return of spontaneous circulation (ROSC). Whether more rapid cooling would further improve survival remains unclear. We sought to determine whether cooling during cardiac arrest before ROSC (i.e., “intra-arrest” hypothermia) has survival benefit over more delayed post-ROSC cooling, using a murine cardiac arrest model.

Methods and Results—A model of potassium-induced cardiac arrest was established in C57BL/6 mice. After 8 minutes of untreated cardiac arrest, resuscitation was attempted with chest compression, ventilation, and intravenous fluid. Mice were randomized to 3 treatment groups (n=10 each): an intra-arrest hypothermia group, in which mice were cooled to 30°C just before attempted resuscitation, and then rewarmed after 1 hour; a post-ROSC hypothermia group, in which mice were kept at 37°C for 20 minutes after successful ROSC and then were cooled to 30°C for 1 hour; and a normothermic control group, in which mice were kept at 37°C. The intra-arrest hypothermia group demonstrated better 72-hour survival than delayed hypothermia and normothermia groups (6/10 versus 1/10 and 1/10 survivors, respectively, P<0.05), with similar differences seen at 6-hour survival and on neurological scoring.

Conclusions—Timing of hypothermia is a crucial determinant of survival in the murine arrest model. Early intra-arrest cooling appears to be significantly better than delayed post-ROSC cooling or normothermic resuscitation. (Circulation. 2004;109:2786-2791.)

Key Words: cardiopulmonary resuscitation • heart arrest • death, sudden • circulation
animals: mice subjected to intra-arrest moderate (30°C) hypothermia, mice subjected to post-ROSC moderate hypothermia, and mice serving as normothermic arrest–resuscitation controls.

Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago (IACUC #71041). General animal husbandry was supervised by the veterinary staff of the Animal Research Center, and long-term survivors were observed under veterinary care on a routine basis.

Animal Preparation

Adult female C57BL/6 mice (weight ~25 to 30 g; Taconic Farms, Germantown, NY) were fasted overnight except for free access to water. Animals were anesthetized with 80 µg/g of ketamine HCl and 12 µg/g xylazine HCl delivered by intraperitoneal injection (pre-mixed formulation; K-113, Sigma). During surgical preparation, body temperature was maintained between 36.5° to 37.5°C by an incandescent heating lamp and monitored by a rectal thermocouple probe (Omega Engineering Inc). The trachea was orally intubated with a 20-gauge catheter (Angiocath, Becton Dickinson) mounted on a blunt needle with a 145° angled tip. The tracheal tube placement was verified and secured, and mechanical ventilation was initiated (Flexivent EC-VF-2, Scireq Scientific Respiratory Equipment Inc). Ventilator settings included a tidal volume of 12.5 µL/g, a respiratory rate of 110 breaths per minute, a FIO2 of 1.0, and a positive end-expiratory pressure of 2 cm H2O. Arterial blood pressure was measured with a Millar solid-state pressure catheter (SPR-671, Millar Instruments Inc) inserted through the right carotid. A saline-filled microcatheter (EZ-1101, BioTime Inc) was inserted into the left jugular vein for fluid administration. Blood pressure and needle-probe ECG monitoring data were recorded with the use of a PC-based data acquisition system (DI-720, DI-205, Windaq, DATAQ Instruments, Inc).

Development of the Murine Cardiac Arrest Model

In pilot experiments to develop the murine cardiac arrest model, we attempted both asphyxial and electrical methods of arrest induction (data not shown). We did not favor asphyxial induction because animals had progressive hypotension for several minutes, making it difficult to identify the precise moment of arrest. We attempted electrical induction of arrest but opted against this method, given the prolonged current requirement to induce lethal arrest, frequent spontaneous ROSC, and the apparent electrical injury this method caused. We decided to induce arrest by using intravenous injection of potassium chloride, which appeared to provoke little injury beyond the cessation of cardiac activity. It also afforded a precise onset of arrest that was easily observed through ECG and blood pressure recording. Illustrative ECG recordings are shown in Figure 1. From pilot experiments, we determined that the mean blood potassium level during arrest was 10.6 ± 0.4 mEq/L and returned to normal levels within 60 minutes of ROSC (n = 3). We therefore suspect that the mechanism of arrest in our model involves cardiac standstill secondary to marked hyperkalemia, which resolves over time during chest compressions and ventilation. Additional preliminary work revealed that animals were easily resuscitated after short durations of cardiac arrest or lower doses of potassium chloride and had progressively increased mortality rates with longer ischemic intervals or increased doses of potassium chloride (data not shown).
Experimental Protocol

Animals were randomized before surgical preparation into 3 groups by the blinded drawing of group assignment slips out of a container (n=10 per group). On each of the 30 slips of paper were written 1 of the 3 experimental groups, and slips were drawn before anesthesia or surgical instrumentation. If an animal required exclusion after randomization because of surgical failure (see below), the chosen slip was returned to the container for rerandomization. The 3 groups included (1) an intra-arrest hypothermia group, in which animals were cooled to 30±0.5°C over 2 to 3 minutes before resuscitation and rewarmed 1 hour later, (2) a post-ROSC hypothermia group, in which induction of hypothermia was delayed 20 minutes after ROSC and maintained at 30±0.5°C for 1 hour, and (3) a normothermia group serving as a control, kept at 37.0±0.5°C throughout the experiment. The depth of hypothermia was chosen on the basis of the desire to test “moderate” hypothermia, defined historically as 28°C to 32°C, a depth that could be easily obtained in our model yet would have a reasonable chance of demonstrating benefit. The choice of a 20-minute delay in cooling was based on the results of Kuboyama et al., demonstrating that a 15-minute delay in hypothermia induction reduced protection in a canine model of arrest, and findings from Markarian et al., showing that cerebral protection was attenuated with a 30-minute delay in cooling in a rat model of cerebral ischemia.

After surgical preparation, animals were observed on the ventilator for 50 minutes after ketamine injection. Animals were excluded before arrest if they had a mean arterial pressure (MAP) of <80 mm Hg or demonstrated surgical bleeding or if surgical instrumentation took longer than 40 minutes. Cardiac arrest was induced by administration of 0.08 μg/g potassium chloride (Sigma) through the jugular catheter, confirmed by loss of arterial pressure and asystolic rhythm. After 8 minutes of cardiac arrest, chest compressions were delivered by finger at a rate of ~400 beats per minute by the same investigator for all animals. Chest compressions were adjusted to provide a uniform rate seen on monitoring and a target aortic diastolic pressure of >20 mm Hg. Hypothermia was induced with a cooling blanket fashioned from an ice water–filled surgical glove, and hypothermia was maintained by careful positioning of the cooling blanket and heating lamp to maintain a temperature of 30±0.5°C during cooling and 37±0.5°C in normothermia. After some experience, small fluctuations in temperature could be tolerated by moving the glove partially on or off the animal, and in this fashion temperature could be tightly controlled. Corrective positioning of the cooling blanket was undertaken if the temperature changed ±0.3°C from target temperature. Return of spontaneous circulation was defined as the return of sinus rhythm with a MAP >40 mm Hg lasting at least 1 minute. Cooling data are shown in Figure 2.

After 2 hours of invasive monitoring, catheters were then removed, wounds were surgically closed, and animals were extubated. The animals were continuously observed under a heating lamp and received an intraperitoneal injection of 1 mL 0.9% saline during the first hour after extubation.

In additional pilot experiments, we wished to evaluate whether rectal monitoring was sufficient to follow animal temperature or whether additional temperature probes would be required. We cooled animals (n=3) by using the technique described above and followed rectal as well as brain temperature, using needle temperature probes inserted into cerebral cortex. The brain and rectal temperatures showed close similarity throughout the protocol (see Figure 3), satisfying us that rectal temperature monitoring would be sufficient.

Neurological Scoring

The mice underwent neurological assessment at 6 hours and then on postarrest days 1, 2, and 3. Neurological function scoring was adapted from a grading system for rats and for mice, as detailed in Table 1. Assessment was performed independently by two investigators. Any discrepancies were resolved by an independent assessment by a third investigator, and the score chosen by the majority was accepted.

Statistical Analysis

Mouse characteristics, including hemodynamic data, are reported as mean±SD. Comparisons between time-based measurements on the same animals and baseline differences between each group were analyzed by means of the unpaired Student’s t test. Survival data were tested for significance by means of χ² analysis. Statistical significance was defined as P<0.05. Statistical computations of the Student’s t test and χ² analysis were performed with the use of Microsoft Excel.

Results

Of 61 mice screened for the protocol, 13 were excluded before or during instrumentation, based on prospective criteria described in the Methods section. An additional 18 mice were excluded before arrest induction during the protocol because of instrumentation or technical failure. No mice were excluded once cardiac arrest was induced. In this fashion, 30 mice were randomized to 3 experimental groups, underwent surgical instrumentation lasting <40 minutes from anesthesia to final cannulation, and finally underwent cardiac arrest of 8 minutes’ duration. Baseline characteristics including animal weight, temperature, heart rate, and blood pressure before arrest were indistinguishable in the three groups. Addition-
ally, cooling rates, resuscitation times, and warming rates did not vary significantly between the groups (Table 2). Interestingly, postarrest blood pressure and heart rate were also similar among all 3 groups and only began to show significant differences at 1 hour, suggesting that the physiological benefit from hypothermia develops over this time period.

Animals were cooled with an ice-water blanket, as described in Methods. We were able to achieve rapid cooling in our model (Figure 2), with approximate cooling rates of 3.2 $\pm$ 1.2°C/min and 3.0 $\pm$ 0.8°C/min in the intra-arrest group and post-ROSC group, respectively ($P = NS$). We were also able to maintain fairly reliable temperature control at 30°C after a short period of minor temperature instability. The rewarming rates for the intra-arrest group and post-ROSC group were 0.6 $\pm$ 0.1°C/min and 0.6 $\pm$ 0.3°C/min, respectively ($P = NS$).

After resuscitation, survival and neurological function were scored among the 3 groups of animals. Survival data are shown in Figure 4. The rate of initial ROSC between groups was statistically indistinguishable. After 6 hours, a statistically significant difference in survival existed between the intra-arrest group and the other 2 groups (7 of 10 survivors in the intra-arrest group versus 3 of 10 in the normothermia group and 1 of 10 in the post-ROSC group; $P < 0.05$ each). This difference was maintained at 72 hours (6 of 10 survivors

### Table 1. Neurological Function Scoring System Used in the Protocol

<table>
<thead>
<tr>
<th>Level of consciousness</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No reaction to pinching of tail</td>
<td>0</td>
</tr>
<tr>
<td>2. Poor response to tail pinch</td>
<td>1</td>
</tr>
<tr>
<td>3. Normal response to tail pinch</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Corneal reflex</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No blinking</td>
<td>0</td>
</tr>
<tr>
<td>2. Sluggish blinking</td>
<td>1</td>
</tr>
<tr>
<td>3. Normal blinking</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respirations</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Irregular breathing pattern</td>
<td>0</td>
</tr>
<tr>
<td>2. Decreased breathing frequency, normal pattern</td>
<td>1</td>
</tr>
<tr>
<td>3. Normal breathing frequency and pattern</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Righting reflex</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No turning attempts</td>
<td>0</td>
</tr>
<tr>
<td>2. Sluggish turning</td>
<td>1</td>
</tr>
<tr>
<td>3. Turns over spontaneously and quickly</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coordination</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No movement</td>
<td>0</td>
</tr>
<tr>
<td>2. Moderate ataxia</td>
<td>1</td>
</tr>
<tr>
<td>3. Normal coordination</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Movement/activity</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No spontaneous movement</td>
<td>0</td>
</tr>
<tr>
<td>2. Sluggish movement</td>
<td>1</td>
</tr>
<tr>
<td>3. Normal movement</td>
<td>2</td>
</tr>
</tbody>
</table>

| Total possible score          | 12    |

### Table 2. Baseline Characteristics of the Three Mouse Groups

<table>
<thead>
<tr>
<th></th>
<th>Normothermic Control (n=10)</th>
<th>Intra-Arrest Cooling (n=10)</th>
<th>Post-ROSC Cooling (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>29.8 $\pm$ 4.2</td>
<td>30.6 $\pm$ 4.1</td>
<td>31.1 $\pm$ 2.5</td>
</tr>
<tr>
<td>Heart rate before arrest, bpm</td>
<td>282 $\pm$ 57</td>
<td>291 $\pm$ 58</td>
<td>295 $\pm$ 46</td>
</tr>
<tr>
<td>MAP before arrest, mm Hg</td>
<td>101 $\pm$ 8</td>
<td>104 $\pm$ 8</td>
<td>101 $\pm$ 7</td>
</tr>
<tr>
<td>Temperature before arrest, °C</td>
<td>36.7 $\pm$ 0.1</td>
<td>36.8 $\pm$ 0.1</td>
<td>36.7 $\pm$ 0.2</td>
</tr>
<tr>
<td>CPR time to ROSC, s*</td>
<td>124 $\pm$ 31</td>
<td>129 $\pm$ 64</td>
<td>144 $\pm$ 44</td>
</tr>
<tr>
<td>Temperature at end of 2-h protocol, °C*</td>
<td>36.7 $\pm$ 0.2</td>
<td>36.8 $\pm$ 0.2</td>
<td>36.6 $\pm$ 0.3</td>
</tr>
<tr>
<td>Cooling rate, °C/min*</td>
<td>NA</td>
<td>3.2 $\pm$ 1.2</td>
<td>3.0 $\pm$ 0.8</td>
</tr>
<tr>
<td>Warming rate, °C/min*</td>
<td>NA</td>
<td>0.6 $\pm$ 0.1</td>
<td>0.6 $\pm$ 0.3</td>
</tr>
<tr>
<td>MAP at ROSC, mm Hg*</td>
<td>82 $\pm$ 18</td>
<td>75 $\pm$ 6</td>
<td>79 $\pm$ 13</td>
</tr>
<tr>
<td>Heart rate at ROSC, bpm*</td>
<td>467 $\pm$ 41</td>
<td>384 $\pm$ 10†</td>
<td>475 $\pm$ 49</td>
</tr>
<tr>
<td>MAP 60 min after ROSC, mm Hg*</td>
<td>48 $\pm$ 23</td>
<td>62 $\pm$ 5†</td>
<td>42 $\pm$ 11</td>
</tr>
<tr>
<td>Heart rate 60 min after ROSC, bpm*</td>
<td>366 $\pm$ 55</td>
<td>470 $\pm$ 12†</td>
<td>403 $\pm$ 48</td>
</tr>
</tbody>
</table>

*Initially n=10 for each group. For these parameters, calculation includes initial survivors only. Thus, normothermic group, n=7; intra-arrest group, n=9; post-ROSC cooling, n=6. NA indicates not applicable.

Differences between groups are not statistically significant unless marked (by †), indicating $P < 0.05$ for intra-arrest group compared with each other group.

**Figure 4.** Survival after cardiac arrest. Kaplan-Meier survival plot of 3 experimental groups is shown. Asterisks denote statistical significance of survival at 6, 18, and 72 hours between the intra-arrest hypothermia group and the other 2 groups, $P < 0.05$ each. Number of animals surviving at 72 hours is shown at right.
in the intra-arrest group versus 1 of 10 in the other two groups; \( P < 0.05 \). All survivors at 72 hours lived indefinitely beyond this time period and were euthanized at 30 days. In prior experiments, control mice undergoing the identical operative procedures and instrumentation without cardiac arrest demonstrated 100% survival at 3 days (n=10; data not shown).

The majority of deaths during our protocol occurred during the first 6 hours after resuscitation, and it is therefore during this time period that hypothermia probably plays a beneficial role. All resuscitated animals survived at least 2 hours, at which point extubation and removal of catheters took place. As shown in Table 2, animals in the two groups with higher death rates also had lower blood pressure compared with the intra-arrest hypothermia group at 1 hour after ROSC, suggestive of a shock-like state. The animals that died during the initial 6 hours had poor respiratory effort and decreased consciousness/neurological performance, also suggestive of shock. No animals died suddenly without these preceding signs and symptoms.

Although neurological evaluation after the protocol revealed excellent function by 72 hours in all survivors (Figure 5), the intra-arrest group demonstrated significantly improved function at 6 hours compared with the normothermia group (9.6±2.1 versus 6.7±2.3, \( P < 0.05 \)). At 18 and 72 hours, this difference between survivors was not statistically significant. However, because few survivors were present in the post-ROSC and normothermia groups, our ability to make a valid comparison is limited.

Discussion

Our data suggest that the induction of intra-arrest hypothermia can confer a long-term survival benefit after resuscitation that is significantly better than 20-minute post-ROSC hypothermia or normothermic resuscitation in a mouse model of 8 minutes of untreated cardiac arrest. In addition, the immediate rates of ROSC were similar in all 3 groups, consistent with the concept that intra-arrest hypothermia reduced deaths that would have occurred later during the postresuscitation period. It is notable that intra-arrest hypothermia to 30°C did not appear to impair resuscitation.

There are two potential explanations for why the 20-minute delay reduced the effectiveness of hypothermia so significantly. First, if cooling is mostly effective during the postresuscitation period, a post-ROSC delay of 20 minutes caused the loss of protection—perhaps a delay of only 2 minutes after ROSC would have ameliorated this loss. Second, it is possible that intra-arrest cooling, resulting in “cool reperfusion,” somehow attenuated damage in these animals. Under this construct, cool reperfusion may be a method for avoidance of lethal reperfusion injury.

The notion of cooling during or even before ischemia is well described in surgical studies, but intra-arrest cooling has been relatively unexplored in the setting of cardiac arrest.9,13–15 The theoretical advantages to cooling before ROSC may include decreasing reperfusion-related injury. Protective cellular mechanisms consistent with this concept include attenuation of the oxidant burst seen within minutes of normothermic reperfusion or the inhibition of reperfusion-activated apoptotic pathways.12 The few studies that attempted intra-arrest hypothermia have demonstrated encouraging protection patterns. Leonov et al13 performed external cranial intra-arrest cooling in a canine model of cardiac arrest, with additional cooling provided by cardiac bypass. Cooling in this combined fashion was more protective than normothermia.

In our model, animals treated with delayed hypothermia or normothermia had markedly increased death rates during the first 6 hours after resuscitation. Although we do not have direct information about the cause of death, animals died slowly in a shock-like state. It is possible that reperfusion injury causes cardiac dysfunction or loss of vascular tone in our animals, which is ameliorated by early application of hypothermia. This may explain the relative hypotension seen in the normothermic and delayed hypothermia groups after 1 hour (see Table 2).

Of particular interest is the fact that in our system, 20-minute post-ROSC cooling showed no advantage over normothermia, although it must be noted that our study was not powered to detect a small benefit. This is in contrast to recent clinical findings but quite consistent with other animal studies.9,16 Kuboyama et al noted a loss of neurological protection after only a 15-minute delay in hypothermia induction in a canine model of cardiac arrest. Loss of protection was also seen after a 30-minute delay in hypothermia induction in a rat model of ischemic stroke.10 Likewise, cellular studies of ischemia followed by reperfusion revealed loss of protection after a 15-minute delay in cooling (Vanden Hoek et al, unpublished data, 2002). The findings from these studies, in fact, guided our choice of a 20-minute cooling delay in our model system. Collectively, these facts suggest that the earlier cooling is achieved in the setting of ischemia/reperfusion, the better the outcome. More specifically, cooling during the intra-arrest period may provide a more pronounced benefit than cooling initiated after resuscitation. However, it remains clear that delayed hypothermia does provide benefit, as shown by recent cardiac arrest trials and in other systems by a number of recent investigations.17,18 The timing of hypothermia will be a critical consideration for future clinical trials.
Finally, we note that our novel mouse model of cardiac arrest has certain limitations. Although two other laboratories have published initial experiments with mouse models of cardiac arrest, the model is relatively new and technically challenging, and its predictive value on human biology remains to be demonstrated. However, despite these limitations, the mouse model appears to be useful for exploring global ischemia/reperfusion mechanisms such as those pathways suggested in the recently described “metabolic phase” of cardiac arrest. The model may be particularly appropriate for defining optimal hypothermia parameters because the model allows for rapid and precise control over body temperature because of small animal size.

In conclusion, we have developed a novel mouse model of cardiac arrest and have demonstrated that in this model, intra-arrest hypothermia significantly reduces mortality rates after cardiac arrest compared with either normothermic resuscitation or resuscitation under delayed hypothermic conditions. Intra-arrest hypothermia, which remains clinically untested, may offer an important survival benefit for patients with cardiac arrest. Future studies will help define the timing of the intra-arrest hypothermia and the genetic and biochemical mechanisms of hypothermic protection.

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