Comparison of the Durations of Mild Therapeutic Hypothermia on Outcome After Cardiopulmonary Resuscitation in the Rat

Sen Ye, MD; Yinlun Weng, MD; Shijie Sun, MD; Wei Chen, MD, PhD; Xiaobo Wu, BME; Zilong Li, MD; Max Harry Weil, MD, PhD; Wanchun Tang, MD

**Background**—Current studies have demonstrated that applying therapeutic hypothermia for 12 to 24 hours after resuscitation from cardiac arrest improves the outcomes of cardiopulmonary resuscitation. The present study investigated whether a shorter duration of therapeutic hypothermia induced quickly and early after resuscitation would provide an equal improvement in the outcomes of cardiopulmonary resuscitation.

**Methods and Results**—Ventricular fibrillation was induced and untreated for 8 minutes in 24 male Sprague-Dawley rats. Defibrillation was attempted after 8 minutes of cardiopulmonary resuscitation. Seven minutes after resuscitation, animals were randomized into 4 groups (n=6 each): normothermic, hypothermic–2 hours, hypothermic–5 hours, and hypothermic–8 hours. Animals in the hypothermic groups received rapid cooling, which was started 7 minutes after restoration of spontaneous circulation and maintained at 33±0.5°C for 2, 5, or 8 hours. Normothermic animals were maintained at 37±0.2°C. All animals were anesthetized and ventilated for 8 hours after resuscitation. Blood temperature was significantly decreased in the hypothermic groups. Postresuscitation myocardial function, neurological deficit scores, and 72-hour survival were significantly better in animals treated with hypothermia regardless of the duration of cooling. However, significantly better postresuscitation tissue microcirculation, myocardial ejection fraction, and neurological deficit scores were observed in the hypothermic–2 hours animals.

**Conclusions**—In a rat model of cardiopulmonary resuscitation, a shorter duration of mild hypothermia induced rapidly and early after restoration of spontaneous circulation improved postresuscitation microcirculation, myocardial and cerebral functions, and survival as well as, or better than, prolonged duration of hypothermia after resuscitation. (Circulation. 2012;125:123-129.)

**Key Words:** cardiac arrest ■ cardiopulmonary resuscitation ■ hypothermia ■ ventricular fibrillation

The current outcomes of cardiopulmonary resuscitation (CPR) are disappointing. A large number of patients die after successful resuscitation or develop permanent severe brain damage, which yields a survival rate of only 4.6%.1,2 During the last decade, both laboratory and clinical studies have demonstrated that actively reducing the blood temperature to 32°C to 34°C after resuscitation significantly improved neurologically favorable survival.3-5 Recent studies further demonstrated that both early application of therapeutic hypothermia and rapid achievement of target cooling temperature were the key factors for improving neurological outcome and survival.3,6 Recent guidelines from the American Heart Association and the European Resuscitation Council recommend that unconscious adult patients achieving restoration of spontaneous circulation (ROSC) after out-of-hospital cardiac arrest be thermally cooled to 32°C to 34°C for 12 to 24 hours when the initial rhythm is ventricular fibrillation (VF).7,8 Under certain conditions, therapeutic hypothermia is also used in the treatment of severe traumatic brain injury, stroke, and myocardial infarction.9-11

**Clinical Perspective on p 129**

However, the adverse effects of therapeutic hypothermia have increasingly been recognized. Those adverse effects include increased coagulation times, shivering, overcooling, pulmonary infections, immunosuppression, and prolonged metabolism of many commonly used drugs.12 In addition, increased nursing labor and costs for management of hypothermia are also issues of concern. Because of these adverse effects and implemental issues, the routine use of therapeutic hypothermia after cardiac arrest remains low in the United States today.13 An improved cooling protocol such as a...
shorter duration may aid in increasing the adoption of this lifesaving intervention.

Most of the neuronal damage after cardiac arrest occurs during the early reperfusion period.14 This reperfusion and reoxygenation injury after reestablishment of systemic perfusion occurs mainly during the first several hours after ROSC.15 Therapeutic hypothermia is believed to confer protection against reperfusion injury by multiple mechanisms, including the suppression of free radicals, enzymes, and excitotoxic and inflammatory reactions, in addition to the direct physical protection of membranes.15

In this study, we investigated whether a shorter duration of therapeutic hypothermia induced quickly and early after resuscitation would provide an improvement equal to the outcomes of CPR after a prolonged duration of hypothermia. If a shorter duration of therapeutic hypothermia would provide the same protection, it would certainly reduce the complexity of applying hypothermia therapy. We hypothesized that in a rat model of CPR, a shorter duration of hypothermia induced rapidly and early after ROSC would be equally effective in improving postresuscitation myocardial and neurological function compared with prolonged duration of hypothermia.

**Methods**

This study was approved by the Institutional Animal Care and Use Committee of the Weill Institute of Critical Care Medicine. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.16,17 Our established rodent model of cardiac arrest and resuscitation was used. Healthy male Sprague-Dawley rats 6 to 8 months of age weighing between 500 and 550 g were supplied by a single-source breeder (Harlan Sprague-Dawley Inc, Livermore, CA), which has consistently supplied healthy animals of relatively uniform age and weight. The normal temperature for this rodent model is from 36.7°C to 38.4°C.

**Animal Preparation**

All animals were fasted overnight except for free access to water. The detailed animal preparation has been published previously.18 Briefly, the animals were anesthetized by intraperitoneal injection of pentobarbital (45 mg/kg). Additional doses (10 mg/kg) were administered at intervals of ~1 hour or when required to maintain anesthesia.

The trachea was orally intubated with a 14-gauge cannula (Abbocath-T; Abbott Hospital, North Chicago, IL). A PE-50 catheter (Becton-Dickinson, Franklin Lakes, NJ) was advanced through the left femoral artery into the descending aorta for measurement of aortic pressure. Another PE-50 catheter was advanced through the left external jugular vein and into the right atrium for measurement of right atrial pressures. Aortic and right atrial pressures were measured with high-sensitivity transducers (model 42584-01; Abbott Critical Care Systems, North Chicago, IL). A thermocouple microprobe, 10 cm in length and 0.5 mm in diameter (9030-12-D-34; Columbus Instruments, Columbus, OH), was inserted into the right femoral artery and advanced into the descending aorta to measure blood temperature. A 3F PE catheter (model C-PMS-301J; Cook Critical Care, Bloomington, IN) was advanced through the right external jugular vein into the right atrium. A precurved guidewire supplied with the catheter was then advanced through the catheter into the right ventricle to induce VF, as confirmed by endocardial ECG. All catheters were flushed intermittently with saline containing 2.5 IU/mL crystalline bovine heparin. A conventional lead II ECG was continuously monitored. The blood temperature for all animals was maintained between 36.8°C and 37.2°C during baseline by a heating lamp.

**Table 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th>Group</th>
<th>Normothermic</th>
<th>Hypothermic−2 Hours</th>
<th>Hypothermic−5 Hours</th>
<th>Hypothermic−8 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>531.5±18.5</td>
<td>528.5±17.0</td>
<td>538.5±9.7</td>
<td>523.8±23.3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>360±16</td>
<td>370±12</td>
<td>355±20</td>
<td>360±9</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>140±7</td>
<td>141±10</td>
<td>136±14</td>
<td>140±4</td>
</tr>
<tr>
<td>RA, mm Hg</td>
<td>1.3±0.1</td>
<td>1.4±0.2</td>
<td>1.3±0.4</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>End-tidal CO₂, mm Hg</td>
<td>40±5</td>
<td>39±4</td>
<td>41±3</td>
<td>42±3</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.9±0.2</td>
<td>37.0±0.1</td>
<td>37.0±0.1</td>
<td>36.9±0.1</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>74±4</td>
<td>71±5</td>
<td>73±4</td>
<td>73±5</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>101±17</td>
<td>109±8</td>
<td>107±9</td>
<td>104±8</td>
</tr>
<tr>
<td>MPI</td>
<td>0.62±0.10</td>
<td>0.63±0.09</td>
<td>0.70±0.08</td>
<td>0.66±0.10</td>
</tr>
<tr>
<td>pH</td>
<td>7.47±0.03</td>
<td>7.49±0.02</td>
<td>7.47±0.01</td>
<td>7.46±0.03</td>
</tr>
</tbody>
</table>

MAP indicates mean aortic pressure; RA, right atrial blood pressure; and MPI, myocardial performance index. Values are presented as mean±SD.

**Table 2. Coronary Perfusion Pressure, Duration of Cardiopulmonary Resuscitation, and Number of Defibrillations**

<table>
<thead>
<tr>
<th></th>
<th>Normothermic</th>
<th>Hypothermic−2 Hours</th>
<th>Hypothermic−5 Hours</th>
<th>Hypothermic−8 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP in PC1, mm Hg</td>
<td>23.3±0.8</td>
<td>23.0±0.9</td>
<td>23.0±1.4</td>
<td>22.3±1.5</td>
</tr>
<tr>
<td>CPP in PC8, mm Hg</td>
<td>22.4±1.1</td>
<td>22.7±1.5</td>
<td>22.2±0.4</td>
<td>22.2±0.8</td>
</tr>
<tr>
<td>Duration of CPR, s</td>
<td>487±16</td>
<td>480±0</td>
<td>487±16</td>
<td>480±0</td>
</tr>
<tr>
<td>DFs, n</td>
<td>2.0±1.7</td>
<td>1.2±0.4</td>
<td>1.7±1.2</td>
<td>1.3±0.8</td>
</tr>
</tbody>
</table>

CPP indicates coronary perfusion pressure; PC1, 1 minute after precordial compression; PC8, 8 minutes after precordial compression; CPR, cardiopulmonary resuscitation; and DFs, defibrillations. Values are presented as mean±SD.
Experimental Procedures

Baseline measurements were obtained 10 minutes before the induction of VF. Mechanical ventilation was established with a tidal volume of 0.60 mL/100 g body weight and a frequency of 100 breaths per minute. The inspired O₂ fraction (Fio₂) was maintained at 0.21. VF was electrically induced with a progressive increase in 60-Hz current to a maximum of 3.5 mA delivered to the right ventricular endocardium. The current flow was continued for 3 minutes to prevent spontaneous defibrillation. Mechanical ventilation was stopped after the onset of VF. Precordial compression was started after 8 minutes of VF. Coincident with the start of precardial compression, the animals were mechanically ventilated at a frequency of 100 breaths per minute and with an Fio₂ of 1.0. Precordial compression was maintained at a rate of 200 per minute and synchronized to provide a compression/ventilation ratio of 2:1 with equal compression-relaxation. The depth of compressions was initially adjusted to maintain a coronary perfusion pressure of 22±2 mm Hg. This typically yielded an end-tidal PCO₂ of 33±0.5°C. Resuscitation was attempted with up to three 2-J countershocks after 8 minutes of CPR. If ROSC was not achieved, a 30-second interval of CPR was performed before a subsequent sequence of up to 3 shocks was attempted. This procedure was repeated for a maximum of 3 cycles. ROSC was defined as the return of supraventricular rhythm with a mean aortic pressure >50 mm Hg for a minimum of 5 minutes.

Seven minutes after successful resuscitation, animals were randomized into 4 groups of 6: normothermic, hypothermic–2 hours, hypothermic–5 hours, and hypothermic–8 hours. In animals of the hypothermic groups, rapid cooling was started after randomization and maintained at 33±0.5°C. Rapid cooling was induced with ice packs and an electric fan. Once the target temperature was reached, it was maintained with a cooling blanket (Blanketrol II; CSZ, Cincinnati, OH) for 2, 5, or 8 hours, followed by a rewarming period of 1°C/h, after which the animals were maintained at 37±0.2°C until the end of the experiment. Normothermic animals were maintained at 37±0.2°C.

After resuscitation, mechanical ventilation was continued with 100% oxygen for 1 hour, with 50% oxygen for the following hour, and then with 21% oxygen for 6 more hours. All catheters, including the endotracheal tube, were removed after the animals had recovered from anesthesia. The animals were continuously observed by the investigators for an additional 2 hours. The animals were then returned to their cages, which were equipped with a heated pet mat (Allied Precision Industries, Inc, Elburn, IL) to maintain the temperature of the cage at 24°C to 26°C. All the animals were closely monitored for 72 hours.

Measurements

Aortic and right atrial pressures, ECG, blood temperature, and end-tidal PCO₂ values were continuously recorded on a personal computer–based data acquisition system supported by WINDAQ software (DATAQ, Akron, OH). Coronary perfusion pressure was calculated as the difference between aortic and time-coincident right atrial pressures measured at the end of each minute of precordial compression.19

At baseline and hourly after ROSC, the buccal microcirculations were visualized with the aid of a side-stream dark-field imaging device (MicroScan; MicroVision Medical Inc, Amsterdam, the Netherlands) with a 5× imaging objective resulting in an on-screen magnification of 276. Three discrete fields were captured with precaution to minimize motion artifacts. Microvasc lar images were recorded on a DVD with a DVD recorder (DMR-EZ47V; Panasonic AVC Networks, Dalian, China). Individual images were analyzed offline for quantifying microcirculatory blood flows (MBFs) by the method of Spronk et al.20 A semiquantitative score in which 0 represents no flow, 1 indicates markedly reduced flow, 2 indicates reduced flow, and 3 represents normal flow was used and calculated for vessels that were <20 μm in diameter, representing primarily the capillaries. In addition, we counted the perfused capillary density by the method of Taccone et al21 as previously described. Vessel size was measured with a micrometer scale superimposed in the video display. All recordings were analyzed by 3 independent observers.

Myocardial function was measured noninvasively at baseline and hourly after ROSC for a total of 8 hours by a Philips ultrasound system (model HDI11Xe; Philips, Andover, MA) with a 12.5-Hz transducer. All measurements, including myocardial performance index and ejection fraction, were reviewed and confirmed separately by 2 investigators. Ejection fraction served as quantitative data of myocardial contractile function. Myocardial performance index is the ratio of the total time spent in isovolumic activity (isovolumic contraction and relaxation times) to the ejection time, which was obtained as both systolic and diastolic functions.22,23

Neurological function was evaluated according to the method of neurological deficit score at 24-hour intervals for a total of 72 hours. The neurological deficits were scored from 0 (no observed neurological deficit) to 500 (death or brain death).24 The neurological deficit scores were examined and confirmed by 2 investigators blinded to the treatment.

Statistical Analyses

Measurements were reported as mean±SD. For measurements among groups, ANOVA and the Scheffe multiple-comparison techniques were used. The outcome differences were analyzed with the Fisher exact test. A value of P<0.05 was regarded as significant.

Results

Thirty four rats were used for this study; 24 completed the study and were included. Ten animals were excluded, including 5 rats that were not resuscitated, thus yielding 80% of the initial survivors, and another 5 rats were...
Discussion

The present study demonstrates that a shorter duration of early hypothermia after ROSC significantly improved microcirculation and myocardial and neurological outcomes compared with prolonged duration of hypothermia in a rat model of CPR. Although 72-hour survival was not significantly different among the hypothermic groups, the hypothermic–2 hours group had a greater 72-hour survival rate than the hypothermic–5 hours and hypothermic–8 hours groups.

In the present study, microcirculation was impaired significantly in all animals after ROSC. However, it was markedly improved 4 hours after resuscitation in the hypothermic–2 hours group. On the contrary, prolonged duration of hypothermia reduced MBF and perfused capillary density significantly, especially after 8 hours of cooling, which reflected that oxygen delivery was decreased at the level of the capillary exchange beds. A possible reason for the changes in MBF and perfused capillary density in the hypothermic groups was vasoconstriction induced by cooling. Thermoregulator vasoconstriction was typically triggered near 34°C to 35°C, resulting in decreased functional capillary density and MBF, which was restored on rewarming.25,26 This explanation was supported in our study by the fact that MBF was

Table 4. Perfused Capillary Density

<table>
<thead>
<tr>
<th>Group</th>
<th>BL, n/mm</th>
<th>At 1 h, n/mm</th>
<th>At 3 h, n/mm</th>
<th>At 4 h, n/mm</th>
<th>At 5 h, n/mm</th>
<th>At 8 h, n/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermic</td>
<td>5.2±0.8</td>
<td>4.4±0.9</td>
<td>4.3±0.6</td>
<td>3.9±0.8</td>
<td>4.4±0.9</td>
<td>4.6±0.7†</td>
</tr>
<tr>
<td>Hypothermic–2 hours</td>
<td>5.5±0.8</td>
<td>4.8±0.3</td>
<td>4.9±0.9</td>
<td>4.6±0.8</td>
<td>4.5±0.6</td>
<td>5.4±0.3</td>
</tr>
<tr>
<td>Hypothermic–5 hours</td>
<td>5.5±0.7</td>
<td>4.5±0.6</td>
<td>4.5±0.9</td>
<td>4.2±0.7</td>
<td>3.7±0.4*</td>
<td>4.0±0.8*</td>
</tr>
<tr>
<td>Hypothermic–8 hours</td>
<td>5.1±0.3</td>
<td>4.3±0.5</td>
<td>4.3±0.6</td>
<td>4.0±0.4</td>
<td>3.7±0.5*</td>
<td>3.4±0.4*</td>
</tr>
</tbody>
</table>

BL indicates baseline. Values are presented as mean±SD.

*P<0.05 versus hypothermic–2 hour group; †P<0.05 versus hypothermic–8 hour group.
decreased during cooling but increased after rewarming in the hypothermic–2 hours group.

During cardiac arrest, oxygen delivery and metabolic substrates were abruptly halted, and metabolites were no longer removed. CPR only partially reversed this process. Inadequate tissue oxygen delivery can persist even after ROSC because of myocardial dysfunction, hemodynamic instability, and microcirculatory failure. Accumulated oxygen debt led to endothelial activation and systemic inflammation and was predictive of subsequent multiple organ failure and death. A recent study further suggested that a proportion of oxygen debt should be repaid as quickly as possible during resuscitation. Although metabolism and oxygen consumption were reduced during cooling, oxygen delivery decreased significantly in prolonged hypothermia after ROSC-impaired oxygen debt repayment. In contrast, a shorter duration of hypothermia improving microcirculation yielded a better outcome of resuscitation.

This is the first study to compare microcirculation and myocardial and neurological function between shorter and prolonged durations of hypothermia in a rat model of CPR.

Table 5. Survival at 72 Hours

<table>
<thead>
<tr>
<th>Group</th>
<th>At 24 h, n/N</th>
<th>At 48 h, n/N</th>
<th>At 72 h, n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermic</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Hypothermic–2 hours</td>
<td>6/6</td>
<td>5/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Hypothermic–5 hours</td>
<td>6/6</td>
<td>2/6</td>
<td>1/6</td>
</tr>
<tr>
<td>Hypothermic–8 hours</td>
<td>5/6</td>
<td>3/6</td>
<td>1/6</td>
</tr>
</tbody>
</table>

Figure 3. Digital photomicrographs of buccal microcirculation after 8 hours of resuscitation in the (A) normothermic group, (B) hypothermic–2 hours group, (C) hypothermic–5 hours group, and (D) hypothermic–8 hours group.

Figure 4. Ejection fraction (%). BL indicates baseline; VF, ventricular defibrillation; PC, precordial compression; and DF, defibrillation. The bar length represents the SD. *P<0.05, **P<0.01 vs normothermic group; #P<0.05, ##P<0.01 vs hypothermic–2 hours group.

Figure 5. Myocardial performance index. BL indicates baseline; VF, ventricular defibrillation; PC, precordial compression; and DF, defibrillation. The bar length represents the SD.* P<0.05, **P<0.01 vs normothermic group; #P<0.05, ##P<0.01 vs hypothermic–2 hours group.

The pathology of ischemia/reperfusion injury can be separated into 2 mechanisms that play out over 2 time windows for hypothermia: hypoxia-induced cellular dysfunction and reperfusion-induced cell death. Clinically, therapeutic hypothermia for 12 to 24 hours induced in the second window has been proven to reduce mortality and to improve functional recovery after cardiac arrest, as has been confirmed by numerous animal studies. However, recent studies demonstrated that early application of therapeutic hypothermia in the first window yields better neurological outcome and survival compared with delayed hypothermia.

The present study further showed that in a rat model of CPR, a shorter duration of hypothermia induced rapidly in the first window after ROSC may be an optimal solution for management of therapeutic hypothermia, which improves the outcome of resuscitation significantly and has the benefit of reducing the complications from and cost of therapeutic hypothermia.

There were certain limitations of our study. First, the small, lissencephalic rodent brain has different rheological and metabolic properties than the complex, comparatively enormous gyrencephalic human brain, and the relative importance of destructive processes may be different in rats compared with humans. Therefore, the outcome of this study in a rat model of CPR remains to be demonstrated in large-animal and clinical studies. In addition, because of the small animal size, this model allows rapid and precise control over body temperature, which is difficult to achieve in clinical practice at present, and it may be particularly appropriate for defining optimal hypothermia parameters. Second, pentobarbital used for anesthesia may have an adverse impact on basic cardiac function. Although there was no difference in the dose of pentobarbital among the 4 groups, hypothermia might reduce
the metabolism of pentobarbital and affect myocardial contractility. Third, both hyperoxia and hypoxia after ROSC may worsen outcomes of CPR.33 In this study, mechanical ventilation and 100% oxygen were continued in the first hour after ROSC without measurement of arterial blood gas, which may affect the outcome of resuscitation. Fourth, cell death after cardiac arrest is a complex process that may be postponed well beyond the expected maturation period. This delay may occur with the use of therapeutic hypothermia.30,34 Thus, 72 hours of observation might not be long enough to evaluate neurological damage after resuscitation.

### Conclusion

The present study using a rat model of CPR demonstrates that a shorter duration of mild hypothermia induced rapidly and early after ROSC improved postresuscitation microcirculation, myocardial and cerebral functions, and survival as well as or better than prolonged duration of hypothermia after resuscitation.

### Sources of Funding

This study was supported by American Heart Association grant 11HRG4870001. The work was performed at the Weil Institute of Critical Care Medicine, Rancho Mirage, CA.

### Disclosures

None.

### References

11. NIH publication 86–32.


**CLINICAL PERSPECTIVE**

Recent guidelines from the American Heart Association and the European Resuscitation Council recommend that unconscious adult patients achieving restoration of spontaneous circulation after out-of-hospital cardiac arrest be therapeutically cooled to 32°C to 34°C for 12 to 24 hours when the initial rhythm is ventricular fibrillation. Recent studies further demonstrated that both early application of therapeutic hypothermia and rapid achievement of target cooling temperature were key factors for improving neurological outcome and survival. The present study in a rat model of cardiopulmonary resuscitation demonstrated that a shorter duration of mild hypothermia induced rapidly and early after restoration of spontaneous circulation improved postresuscitation microcirculation, myocardial and cerebral functions, and survival as well as, or better than, prolonged duration of hypothermia. Therefore, a shorter duration of hypothermia induced rapidly and early after restoration of spontaneous circulation may be an optimal solution for therapeutic hypothermia that improves the outcome of resuscitation significantly and reduces the complications from and cost of therapeutic hypothermia.
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