Critical Time Window for Intra-Arrest Cooling With Cold Saline Flush in a Dog Model of Cardiopulmonary Resuscitation

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Background—Mild hypothermia improves outcome when induced after cardiac arrest in humans. Recent studies in both dogs and mice suggest that induction of mild hypothermia during cardiopulmonary resuscitation (CPR) greatly enhances its efficacy. In this study, we evaluate the time window for the beneficial effect of intra-arrest cooling in the setting of prolonged CPR in a clinically relevant large-animal model.

Methods and Results—Seventeen dogs had ventricular fibrillation cardiac arrest no flow of 3 minutes, followed by 7 minutes of CPR basic life support and 50 minutes of advanced life support. In the early hypothermia group (n=9), mild hypothermia (34°C) was induced with an intravenous fluid bolus flush and venovenous blood shunt cooling after 10 minutes of ventricular fibrillation. In the delayed hypothermia group (n=8), hypothermia was induced at ventricular fibrillation 20 minutes. After 60 minutes of ventricular fibrillation, restoration of spontaneous circulation was achieved with cardiopulmonary bypass for 4 hours, and intensive care was given for 96 hours. In the early hypothermia group, 7 of 9 dogs survived to 96 hours, 5 with good neurological outcome. In contrast, 7 of 8 dogs in the delayed hypothermia group died within 37 hours with multiple organ failure (P=0.012).

Conclusions—Early application of mild hypothermia with cold saline during prolonged CPR enables intact survival. Delay in the induction of mild hypothermia in this setting markedly reduces its efficacy. Our data suggest that if mild hypothermia is used during CPR, it should be applied as early as possible. (Circulation. 2006;113:2690-2696.)

Key Words: cardiopulmonary resuscitation ■ cooling ■ heart arrest ■ hypothermia ■ resuscitation

After successful resuscitation from cardiac arrest (CA), hypothermia has been shown in several experimental studies to improve cerebral outcome.1–5 On the basis of recent clinical studies, therapeutic mild hypothermia is recommended by the American Heart Association and the International Liaison Committee on Resuscitation for Unconscious Survivors of CA.6,7 Despite a relatively late and slow surface cooling technique, these clinical trials in Europe and Australia documented neurological benefits with mild hypothermia in survivors of out-of-hospital CA.8,9 Because evidence exists that a delay in cooling negates the beneficial effect of mild hypothermia,4,10 some have suggested that hypothermia should be initiated as soon as possible after resuscitation or, preferably, during cardiopulmonary resuscitation (CPR) attempts.5,10 In a recent study of CA in mice, application of mild hypothermia during CPR was shown to enhance outcome compared with its application after restoration of spontaneous circulation (ROSC).11 Similarly, in a clinically relevant study of prolonged ventricular fibrillation (VF) in dogs, we documented that mild or moderate hypothermia induced during 40 minutes of CPR attempts preserves organ viability and significantly improves outcome.12 Intact survival was achieved despite 40 minutes of VF, indicating that effective closed-chest CPR with mild hypothermia opens a therapeutic window of at least 40 minutes for the institution of advanced techniques to restore spontaneous circulation (including cardiopulmonary bypass [CPB]). In clinical cases, however, >60 minutes often is required to initiate CPB.13–15 In the present study, therefore, we sought to examine the time window for successful application of mild hypothermia during 60 minutes of CPR-resistant VF. We hypothesized that in contrast to delayed induction of hypothermia, early application of mild hypothermia minimizes organ injury during prolonged CPR and enables intact survival.

Methods
The experimental protocol was approved by the Institutional Animal Care and Use committees of the University of Pittsburgh. All surgery

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was performed in our animal intensive care unit (ICU) with sterile techniques.\textsuperscript{16,17}

**Protocol**

The protocol (Figure 1) simulated nonresponsive VF CA that was “bridged” during closed-chest CPR of 60 minutes from collapse via transport to initiation of CPB in the hospital emergency department.\textsuperscript{18–20} Seventeen custom-bred hunting dogs (body weight, 19 to 25 kg; age, 8 to 12 months; Pat’s Pine Tree Farm, Williford, Ark) were used. All dogs received the same standardized anesthesia and instrumentation, as described previously.\textsuperscript{18–20} Briefly, anesthesia was induced and maintained with titrated doses of halothane in N\textsubscript{2}O/O\textsubscript{2} 50%/50%. Gastric and bladder catheters and temperature probes were inserted, and the tympanic membrane temperature (T\textsubscript{ty}) was controlled at 37.5±0.1°C with heating blankets and heating lamps. Catheters were inserted into the left femoral artery for monitoring of arterial pressure and blood sampling and into the right femoral artery for later CPB. A pulmonary artery catheter was inserted into the left femoral vein and advanced into wedge position. For rapidly controlling T\textsubscript{ty}, a venovenous extracorporeal shunt cooling system was used.\textsuperscript{21} A 13F catheter was inserted into the femoral vein 20 cm into the inferior vena cava and connected to a 15-m-long tubing (3-mm inner diameter; primed with 120 mL isotonic saline and 500 IU heparin) immersed in ice water. There was no additional systemic heparinization. A shunt flow of 10 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} by a roller pump returned the cooled blood via the right external jugular vein into the superior vena cava using a multiple-holed 19F catheter.

After baseline measurements, the dogs were weaned to spontaneous breathing, and VF was induced with a 95-V AC, 60-Hz transthoracic shock of 2 seconds using subcutaneous needles. Pulselessness was allowed to persist for 3 minutes before initiation of CPR. CPR basic life support (BLS) was then begun with left parasternal chest compressions (dogs were placed supine, then turned 25\% to the right to exert pressure over the heart) at a rate of 80 compressions per minute with a mechanical thumper (Michigan Instruments, Grand Rapids, Mich) and bag ventilation (Fi\textsubscript{o2}=0.21) at a ratio of 5 compressions to 1 ventilation. After 7 minutes of BLS (10 minutes of VF), the dogs were randomized into 2 treatment groups. In the early hypothermia (EH) group (n=8), cooling was initiated at 10 minutes of VF, together with the advanced life support (ALS), simulating immediate cooling by the paramedics. Fi\textsubscript{o2} was increased to 1.0, and 20-\mu g/kg IV boluses of epinephrine were given at 5-minute intervals. Three external transthoracic DC countershocks of 50 J (purposely low to fail to defibrillate) were delivered in rapid sequence. Cooling was induced with a bolus of 20 mL/kg of normal saline at 2°C into the superior vena cava, followed by venovenous extracorporeal pumping at 10 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} until a T\textsubscript{ty} of 34°C was achieved. The dogs were purposefully maintained in VF for a total of 60 minutes. The delayed hypothermia (DH) group (n=8) was subjected to the same insult, but ALS was provided for another 10 minutes of normothermic VF before cooling was induced. Reperfusion after 60 minutes of VF was achieved with CPB, as described previously.\textsuperscript{18–20} The CPB system included a centrifugal pump (Biomedicus, Eden Prairie, Minn), a hollow-fiber membrane oxygenator, and a heat exchanger unit (Medtronic, Anaheim, Calif) and was primed with 10% dextran 40 in isotonic saline:Ringer’s solution (1:1), with 2 mL/kg sodium bicarbonate and 500 U heparin. Flow of 100% O\textsubscript{2} through the oxygenator was adjusted to keep Pa\textsubscript{CO2} at 30 to 35 mm Hg. The CPB flow rate was kept at 100 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} until 120 minutes, at which time it was reduced to 50 mL·kg\textsuperscript{-1}·min\textsuperscript{-1} until the dogs were weaned from CPB at 4 hours. After 15 minutes of recirculation with CPB, DC countershocks of 150 J were delivered; they were increased if needed by 50 J for repeated shocks. If necessary for ROSC, epinephrine 5 \mu g/kg IV was administered and repeated as needed. After ROSC, a norepinephrine infusion was started and titrated to maintain the mean arterial pressure at 90 to 120 mm Hg. To comply with current International Liaison Committee on Resuscitation guidelines, T\textsubscript{ty} of 34°C was maintained by external means (application of ice, warming/cooling blankets, and heating lamps) until 12 hours in both groups. Controlled ventilation was continued to 48 hours, and intensive care was provided until 96 hours or earlier death. Analgesia and sedation were provided with N\textsubscript{2}O/O\textsubscript{2} 50%/50% and boluses of morphine (0.1 to 0.3 mg/kg IV) and diazepam (0.1 to 0.3 mg/kg IV) as needed. At 44 to 48 hours, the dogs were weaned from controlled ventilation and transferred to a step-down ICU, where they were monitored until 96 hours.

**Outcome Evaluation**

Performance was evaluated according to overall performance categories (OPC; 1=normal, 2=moderate disability, 3=severe disability but conscious, 4=coma, and 5=death).\textsuperscript{16,18–20} Neurological function was evaluated as neurological deficit scores (NDS; 0% to 10%=normal, 100%=brain death).\textsuperscript{16–21} OPC and NDS were evaluated by 3 independent observers (not blinded) every 8 hours after extubation to obtain best and final values. If different scores were assigned by the observers, an independent blinded physician was asked to evaluate the performance and neurological outcome of the dogs. After final evaluation at 96 hours, the dogs were reanesthetized for morphological studies and brain perfusion fixation for histological damage scoring.\textsuperscript{16,17} A pathologist, blinded to treatment, scored 19 distinct anatomic brain regions for severity and extent of ischemic neuronal changes, infarcts, and edema.\textsuperscript{22} A total brain histological damage score (HDS) >40 represents moderate damage, and HDS >100 represents severe damage. Myocardial injury was quantified sepa-
rately in the right and left ventricles. The degree of myocardial damage was scored from 0 to 5 (0 = absent, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe) on the basis of the size and anatomic distribution (endocardial, epicardial, or transmural). The myocardial lesions also were scored for pattern (1 = focal, 2 = multifocal, 3 = focally extensive, 4 = diffuse), for anatomic location (1 = primarily endocardial, 2 = primarily epicardial, 3 = extensively endocardial and epicardial, 4 = transmural), and for appearance (1 = exclusively pale, 2 = mottled pale and hemorrhagic). The total myocardial damage score was the indexed sum of all the scores (0 = no damage, 100 = severe damage).

Statistical Analysis
Repeated-measures ANOVA was performed, followed by Bonferroni/Dunn post hoc tests to identify differences in hemodynamic and arterial blood gas parameters between groups over time. The Fisher exact test was used to assess differences in survival and OPC proportions (dichotomized to OPC 1 and 2 = good outcome; OPC 3, 4, or death = bad outcome) between groups. Resuscitation variables, HDS, and myocardial damage scores were analyzed with the Mann-Whitney U test. A value of \( P < 0.05 \) was considered statistically significant.

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

Results
Sixteen of 17 dogs remained in protocol for the duration of study. One dog in the EH group was excluded because of accidental extubation leading to CA in the ICU. There were no group differences in body weight or in baseline values of any observed or controlled variables.

Temperature (Figure 2) and blood pressures (Figure 3) changed as expected, according to protocol. Changes in arterial pH, serum sodium and potassium concentrations, and norepinephrine infusion rate are summarized in Figure 4. Resuscitation variables are summarized in the Table. ROSC was achieved after 15 to 235 minutes of CPB. All but 1 dog in the EH group survived to 96 hours. In contrast, all but 1 dog in the DH group died within 37 hours with multiple organ failure, including cardiovascular failure (\( P = 0.01 \)).

During closed-chest CPR, mean arterial blood pressure was 45±4 mm Hg, with a diastolic pressure of 24±5 mm Hg, without overall group differences (Figure 3). These pressures increased in both groups transiently after the intravenous flush of cold normal saline at 10 or 20 minutes of VF, respectively, and returned to preinfusion levels within 3 minutes, without statistical differences between groups. Venovenous shunt cooling was initiated in both groups with an intravenous saline flush, which decreased Tty from 37.5°C to 35.8±1.2°C (Figure 2). Tty of
34°C was achieved at 6.0±2.7 minutes after the initiation of cooling (3.5 minutes after the start of venovenous shunt cooling) in both groups (after 16.6 and 25.4 minutes of VF in the EH and DH groups, respectively).

In the DH group, unstoppable deterioration after ROSC was due to extracerebral organ failure. In 4 dogs, increased microvascular fluid extravasation resulting in general edema, ascites, and pleural effusion was followed by vasopressor-resistant shock. Oliguria or anuria also was observed in 2 of these dogs, despite normal central venous and pulmonary artery wedge pressures. Two dogs in the DH group developed vasopressor-resistant shock and were euthanized at 27 and 36 hours, respectively; their brains were processed for histological scoring. The 3 remaining dogs died in secondary VF that was resistant to vigorous CPR with antiarrhythmic treatment and repeated counter-shocks. The only nonsurviving dog in the EH group died with pulmonary edema and hemoptysis.

Myocardial necrosis was present in all dogs in both groups despite patent coronary arteries. Superficial subendocardial hemorrhage and papillary muscle necrosis were observed in both ventricles in 2 dogs in the EH group and in 6 of 8 dogs in the DH group (P=NS). Milder lesions, consisting of focal areas of subendocardial or subepicardial infarctions, and focally extensive hemorrhagic lesions were present in all dogs, resulting in a total myocardial damage score of 58.5 (range, 43 to 93) in the EH group and 68.5 (range, 47 to 93) in the DH group (P=NS).

In the EH group, 5 of 7 surviving dogs were functionally normal (OPC 1 or 2), 1 had OPC 3, and 1 had OPC 4 (coma) at 96 hours of recovery. Histologically, 4 of 8 dogs in this group were normal (HDS 0), 1 had HDS 16, 1 had 22, and 1 had 98. The only surviving dog in the DH group was functionally normal at 96 hours (OPC 1, NDS 0) and had an HDS of 32 (mild injury) (Figure 5). Because of early deaths, only 2 additional brains in this group could be studied histologically. One had an HDS score of 38 and the other had 45 (Figure 5). Histopathological changes consisted mainly of focal infarctions and scattered ischemic neurons in the frontal, parietal, and temporal cortices, as well as multifocal gliosis and vasculitis involving primarily the basal ganglia.

**Discussion**

In the present study, we demonstrated that early induction of mild hypothermia preserves the organism in a scenario modeling prolonged “unsuccessful” CPR attempts, enabling intact survival after up to 60 minutes of VF. Importantly, a 20-minute versus 10-minute delay in cooling negates the beneficial effects of hypothermia in this model of prolonged VF CA.

The benefit derived from mild hypothermia after ROSC for cerebral recovery has been well documented.1–5,8,9 Using a mouse model of CA, Abella et al recently reported improved outcome when cooling was induced during CA but not after ROSC. Similarly, in a clinically relevant large-animal model of CA, we reported survival with full neurological recovery after 40 minutes of VF if mild or moderate hypothermia was initiated during ROSC.
attempts, but not after ROSC.\textsuperscript{12} Cooling was started 20 minutes after normothermic CA (3 minutes of no flow, 7 minutes of BLS, and 10 minutes of ALS), simulating the time required for the ambulance to arrive and for the paramedics to attempt ROSC. Our present study is based on these 2 reports and suggests a critical time window to maximize what can be a dramatic benefit of mild hypothermia in this setting. We believe that our data may have broad implications for the potential application of mild cooling during CPR. Moreover, our data suggest that for mild hypothermia to be maximally effective, it should probably be applied as early as possible. Future clinical trials will need to address the safety and efficacy of mild cooling during CPR.

In a recent study in patients with acute myocardial infarction, Ly et al\textsuperscript{23} failed to document hemodynamic instability or arrhythmias when hypothermia was induced before reperfusion therapy. Recent studies also have shown the benefit of mild and moderate hypothermia on defibrillation success during VF.\textsuperscript{24,25} However, it is not clear if there is a critical time window for this additional benefit of intra-arrest hypothermia with regard to defibrillation success. Nevertheless, the use of mild or moderate hypothermia would be expected to facilitate rather than reduce defibrillation success. Thus, our work also builds on these important parallel findings.

Our study could also have potential implications for situations in which CPB is attempted to achieve ROSC after prolonged CPR. Clinical studies indicate that, even for witnessed in-hospital CA victims, up to 1 hour often is required for cannulation and implementation of CPB.\textsuperscript{13–15} These studies also have shown that a delay >30 minutes in the initiation of CPB can lead to poor outcome despite vigorous CPR. Therefore, we aimed to explore whether mild hypothermia would enable intact survival after 60 minutes of VF, giving a considerable window of time for transportation of the CA victim and implementation of CPB. Our results support this hypothesis and indicate that cooling should be initiated as early as possible to achieve this goal.

The fluid load (20 mL/kg) used to initiate the cooling also may affect the chance of survival by altering the blood rheology and improving myocardial and cerebral blood flow. A recent study by Bernard et al\textsuperscript{26} demonstrated that a fluid bolus of 30 mL/kg ice-cold lactated Ringer’s solution in comatose survivors of CA decreased core temperature by 1.6°C over 25 minutes and improved blood pressure. Nordmark et al\textsuperscript{27} also reported a 1.6°C decrease in core body temperature using 30 mL/kg infusion of acetated Ringer’s solution at 4°C in pigs. Thus, in the present study, an earlier augmentation of the organ blood flow during the low-flow state of CPR may have resulted in an improved outcome in the EH group. To differentiate between the effects of EH and early fluid load, additional studies are needed with normothermic flush at 10 minutes of VF but cooling at 20 minutes of VF. In additional experiments at our laboratory (n = 2), however, early volume expansion with delayed cooling did not result in good outcome: Both dogs died within 26 hours after ROSC in vasopressor-resistant shock. Prior reports on the role of fluid boluses during CPR similarly suggest that the benefit observed in the present study is due more likely to cooling than to volume expansion.\textsuperscript{28} These findings support the hypothesis that good outcome in the EH group is, in fact, the result of EH and not a single-volume bolus during CPR. Nevertheless, we cannot rule out a possible synergistic effect of hypothermia and volume administration.

The unexpected failure of EH to reduce myocardial damage scores may be attributed to the difference in survival time between groups, which alters the evolution of histological changes after myocardial ischemia and reperfusion injury.\textsuperscript{29} Alternatively, this finding could suggest that EH influences myocardial function more than its morphological outcome in this paradigm. One other possibility is that myocardial protection would be enhanced with either a lower target temperature or longer post-ROSC application of mild cooling. Ao et al\textsuperscript{30} reported histological myocardial protection with 20 hours of mild cooling in a 15-minute VF model, followed by extracorporeal support. Likewise, in a rabbit model of myocardial infarction, Hale et al\textsuperscript{31} reported myocardial protection with regional hypothermia induced early after ischemia. These findings parallel our recent work showing that sustained mild cooling further enhances neuronal protection after CA.\textsuperscript{32} Furthermore, we cannot rule out the possible interaction between central nervous system injury and multiple organ failure, with EH indirectly improving survival by mitigating central nervous system injury. We also recog-
nize the limitation that our model does not incorporate coronary artery disease, which often complicates refractory CA. Further studies of the specific mechanisms underlying the potential benefits of early application of mild hypothermia during CPR are needed.

We recognize that the effect of hypothermia on the capability of achieving ROSC requires further studies. Our findings, however, magnify the importance of those studies because earlier application of mild hypothermia during CPR was dramatically effective. For CPR-resistant cases, nevertheless, the authors suggest that mild hypothermia should be considered as soon as possible during CPR in cases in which a bridge to prolonged CPB for delayed resuscitation is being considered. Early, but not delayed, hypothermia enables intact survival after prolonged non-responsive VF CA in our model.

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References
Clinical Perspective

Innovative clinical approaches are needed to improve the poor prognosis after cardiac arrest (CA). Mild therapeutic hypothermia is promising. Based on the evidence evaluation from the 2005 International Consensus Conference on Cardiopulmonary Resuscitation, the International Liaison Committee on Resuscitation recently recommended avoiding active rewarming of CA survivors and instead actively inducing mild hypothermia in hemodynamically stable comatose survivors. During circulatory arrest for cardiac surgery, protective hypothermia (induced before circulatory arrest) is considered the single most important measure to minimize ischemic brain injury. Similarly, CA victims could potentially benefit from an earlier induction of hypothermia during CA (intra-arrest cooling). In the present study, we show that there is a critical time window for intra-arrest hypothermic protection in a canine model of CA treated with cardiopulmonary resuscitation. Early (but not late) intra-arrest cooling by infusion of cooled saline reduced neurological injury and enabled intact survival from prolonged CA. These findings suggest that hypothermia initiated early during resuscitation may improve CA outcomes and merits clinical testing. In addition, when CA is resistant to standard resuscitative measures, early intra-arrest cooling may delay neurological injury sufficiently to warrant implementation of cardiopulmonary bypass or other mechanical support for more arrest victims.
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