Optimal Protective Hypothermia in Arrested Mammalian Hearts

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Many therapeutic hypothermia recommendations have been reported, but the information supporting them is sparse, and reveals a need for the data of target therapeutic hypothermia (TTH) from well-controlled experiments. The core temperature $\leq 35^{\circ}\text{C}$ is considered as hypothermia, and 29°C is a cooling injury threshold in pig heart *in vivo*. Thus, an optimal protective hypothermia (OPH) should be in the range $29\text{--}35^{\circ}\text{C}$. This study was conducted with a pig cardiopulmonary bypass preparation to decrease the core temperature to $29\text{--}35^{\circ}\text{C}$ range at 20 minutes before and 60 minutes during heart arrest. The left ventricular (LV) developed pressure, maximum of the first derivative of LV ($\text{dP/dt}_{\text{max}}$), cardiac power, heart rate, cardiac output, and myocardial velocity (V_{max}) were recorded continuously via an LV pressure catheter and an aortic flow probe. At 20 minutes of off-pump during reperfusion after 60 minutes arrest, 17 hypothermic hearts showed that the recovery of V_{max} and $\text{dP/dt}_{\text{max}}$ established sigmoid curves that consisted of two plateaus: a good recovery plateau at $29\text{--}30.5^{\circ}\text{C}$, the function recovered to baseline level (BL) ($V_{\text{max}} = 118.4\% \pm 3.9\%$ of BL, LV $\text{dP/dt}_{\text{max}} = 120.7\% \pm 3.1\%$ of BL, $n=6$); another poor recovery plateau at $34\text{--}35^{\circ}\text{C}$ ($V_{\text{max}} = 60.2\% \pm 2.8\%$ of BL, LV $\text{dP/dt}_{\text{max}} = 28.0\% \pm 5.9\%$ of BL, $p < 0.05$, $n=6$), which are similar to the four normothermia arrest (37°C) hearts ($V_{\text{max}} = 55.9\% \pm 4.8\%$ of BL, LV $\text{dP/dt}_{\text{max}} = 24.5\% \pm 2.1\%$ of BL, $n=4$). The $32\text{--}32.5^{\circ}\text{C}$ arrest hearts showed moderate recovery ($n=5$). A point of inflection (around $30.5\text{--}31^{\circ}\text{C}$) existed at the edge of a good recovery plateau followed by a steep slope. The point presented an OPH that should be the TTH. The results are concordant with data in the mammalian hearts, suggesting that the TTH should be initiated to cool core temperature at 31°C .

Introduction

THE MORTALITY RATE AFTER RESUSCITATION from out-of-hospital cardiac arrest is high. There are significant variations between regions and individual institutions (Stub *et al.*, 2012). Many guidelines, such as International Liaison Committee on Resuscitation (ILCOR), Cardiopulmonary Resuscitation (CPR) Guideline, and European Resuscitation Council (ERC), have recommended therapeutic hypothermia for postcardiac arrest syndrome as a beneficial and effective treatment (International Liaison Committee on Resuscitation, 2006; Mancini, 2011; National Institute for Health and

Clinical Excellence, 2011; Boyce *et al.*, 2012; Bro-Jeppesen *et al.*, 2012; Jahn *et al.*, 2012; Nagao, 2012; Gibson and Andrews, 2013; Søreide *et al.*, 2013; Vollroth *et al.*, 2013). The survival depends on multiplicands, including guideline quality, the levels of education, local implementation, and so on (Zobel *et al.*, 2012). Recently, $32\text{--}34^{\circ}\text{C}$ has been commonly used as the target hypothermia (Don *et al.*, 2009; Kupchik, 2009; Gibson and Andrews, 2013); $32\text{--}33^{\circ}\text{C}$ (Vollroth *et al.*, 2013), 32.5°C (Murtha *et al.*, 2014), $32.5\text{--}33.5^{\circ}\text{C}$ (Bro-Jeppesen *et al.*, 2012), 33°C (Zobel *et al.*, 2012), 34°C (Choi *et al.*, 2013; Testori *et al.*, 2013), and $33\text{--}36^{\circ}\text{C}$ (Nielsen *et al.*, 2013) are also used. The results are different.

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Further studies need to define an optimal protective hypothermia (OPH) for a successful resuscitation. A target therapeutic hypothermia (TTH) consists of OPH with fewer side effects.

In the beating pig hearts *in vivo*, there existed a cooling injury threshold for contractility at 29°C (Ning *et al.*, 2008), suggesting that OPH for myocardial protection should be $\geq 29^\circ\text{C}$. However, based on reported different protocol treatments, it is difficult to accurately determine an OPH. It is necessary to explore OPH with well-controlled experiments. A series of studies on mammalian isolated hearts have observed that treatment with 29–30.5°C showed obvious protection after arrest, but 34°C and higher has less protection (Xu *et al.*, 1996; Ning *et al.*, 1998a, 1998b, 1998c, 2007b), suggesting that an OPH for myocardial protection should be $< 34^\circ\text{C}$. The aim of this study was to clarify an OPH in the pig hearts *in vivo* with a clinical surgery used method—cardiopulmonary bypass (CPB) treated by cardiac arrest for 60 minutes. CPB has also been used in many animal models. We would like to emphasize that in this study all experimental conditions were controlled rigorously so that the only variable between the experiments was temperature. This study indicates that the OPH is similar to previous studies in the isolated rabbit hearts (29–30.5°C). This is also concordant with the patient who suffered from sudden cardiac arrest and was treated with hypothermia rescue (32°C central catheter), resulting in full recovery.

Materials and Methods

The object treatment and experimental procedures were in accordance with institutional and NIH guidelines. The pig model with CPB was used to explore the OPH *in vivo*.

Pig surgical procedures

Pigs (age 25–30 days, 11–15 kg, male) received an intramuscular injection of ketamine (33 mg/kg), atropine (0.02 mg/kg), and xylazine (2 mg/kg) for sedation. Then, under propofol (3 mg/kg IV for initial and 3 mg/kg/h for maintenance) anesthesia, the pigs were intubated and ventilated with 40% oxygen-air mixed gas. The cannulas were inserted to femoral artery and veins were separated to monitor blood pressure, to collect blood samples, and to infuse propofol and 5% dextrose-9% NaCl solution. Blood oxygen and CO₂ levels and pH were measured with Radiometer ABL 820. A midline sternotomy was performed to open the chest. A flow probe was placed at the ascending aorta to measure cardiac output (CO) (TS420; Transonic Systems, Inc.). A Millar high-fidelity micro manometer was passed into the left ventricle via the apex to measure left ventricular pressure (LVP). A catheter was placed at the root of the aorta via left carotid artery to inject cardioplegic solution. Umbilical tape was placed around the ascending aorta just below the flow probe as a snare for later aortic occlusion. The cannulas were inserted into the aorta and right atrial appendage to institute CPB via a heart-lung machine. The extracorporeal circuit and assisted circulation during aortic cross-clamping is commonly used in the surgery. It can be controlled in a stable condition to compare the changes at different temperature treatments. A Hollow Fiber Oxygenator (Minimax X Plus; Medtronic) was connected in the circle. The circuit was primed with 500 mL of 10% Dextran 40–0.9% NaCl solution to establish a CPB. The circuit heat exchanger and a heating

pad under the pig were maintained at 37°C during the normothermic period. All the hemodynamic probes were connected to a PowerLab 16/30 recorder (AD Instruments) and an online computer. LVP, CO, heart rate (HR), maximum of the positive and negative first derivative of the left ventricle ($\pm dP/dt_{\max}$), cardiac power (CP), after load (peripheral resistance, mean arterial blood pressure, and BPmean), and preload (the left ventricular end diastolic pressure [LVEDP]) were maintained in the physiological range (< 12 mmHg) in all animals. The developed pressure ($DP = LVP$ peak value – LVEDP) was continuously recorded. The hemodynamic indices should be stable and kept in the normal physiological ranges (systolic blood pressure > 80 mmHg, $dP/dt_{\max} > 1500$ mmHg/s, HR > 100 beats/min) as the physiological condition. If a pig could not reach the normal stable condition, it would be excluded in this study.

Pig cardiac arrest

Since low temperature can prolong cardiac contractions and slow down the high-energy phosphate depletion during arrest, it is necessary to use cardioplegia to stop the heart immediately, thus to remove discrepancies in myocardial performance and energy level between the designed different temperature treatments (Ning *et al.*, 1998b). Cardiac arrest was induced by an injection of Plasma-Lyte A solution (Baxter), containing (in mEq/L) NaCl 140, MgCl₂ 3, acetate 27, glucose 23, and KCl added to 45. When the aorta was occluded, cardioplegia solution was injected to arrest the heart (1 mL/s, total of 0.5 mL/kg). At the end of 60 minutes arrest, the hearts were reperfused on-pump for 20 minutes. Defibrillation was used, if ventricular fibrillation appeared, and could not be controlled back to normal rhythm by finger patting. After 20 minutes of reperfusion, the pump was turned off. Off-pump recording was continued for 40 minutes.

Pig hypothermia protocol

During the heart-lung machine on-pump period, the levels of LVP, LVEDP, and CO were controlled to match the levels as before on-pump. After 10 minutes of stable on-pump, hemodynamic indices and blood gas levels were measured and should be similar to the physiological baseline condition and took it as the “baseline level” (BL). Then, the hypothermia protocol was initiated.

Under anesthesia and open chest condition, 37°C was maintained stable and monitored by thermal probes in esophagus and rectum. Thirty-seven degree celsius was taken as the normothermia condition. A previous study indicated that as the pig heart temperature decreased from 37°C to 35.5°C, only the HR decreased progressively (Ning *et al.*, 2008), but DP, $dP/dt_{\max}$, and CO did not significantly change. Generally, a core temperature $\leq 35^\circ\text{C}$ is considered as hypothermia (Papile, 2005; Gibson and Andrews, 2013). In the beating pig hearts *in vivo*, there existed a cooling injury threshold for contractility at 29°C (Ning *et al.*, 2008), suggesting that an optimal hypothermia for myocardial protection should be $\geq 29^\circ\text{C}$. The determination of target temperature was created by decreasing the core temperature; according to the golden section coefficient 0.618. We designed to explore the optimal hypothermia between 29°C and 35°C in 17 hearts. Experiments were divided into 3 groups: 29–30.5°C, 32–32.5°C, and 34–35°C. The normothermia (37°C) arrest hearts were

taken as controls (four hearts) to be compared with the hypothermia arrest hearts.

The cardioplegia solution was injected to arrest the heart for 60 minutes. At the end of the arrest, the hearts were reperfused on-pump for 20 minutes. The hearts were rewarmed to 35.5°C within 15 minutes and returned to 37°C within 20 minutes. Then, the pump was turned off. Off-pump recording was continued for 40 minutes. The heating pad was turned off during the period of hypothermia and turned on during reperfusion. Previous data showed that preischemic hypothermia could provide cardiac protection (Ning *et al.*, 1998b). Adding 20 minutes of hypothermia before the hypothermic arrest showed very good recovery during reperfusion in the isolated hearts (Ning *et al.*, 2007b). In the cardiac surgery, hypothermia could be started before heart arrest. Therefore, we designed to start hypothermia 20 minutes before heart arrest. Except changes in temperature, the protocol is the same in all groups, so that different effects only depend on the temperature.

To determine OPH. Myocardial injury reflects the decrease in contractility. To present initial velocity of myocardial contraction, besides the dP/dt_{max} , a related index is usually used. It is defined as $V_{max} = LV\ dP/dt_{max} / LVP_{max}$ and high correlation with cardiac contractility (homeometric autoregulation, and inotropic effect) (Higgins and Bookstein, 1977; Ning and Zhou, 1979; Carey *et al.*, 2003; Hörburger *et al.*, 2012; Perlman *et al.*, 2012; Ning *et al.*, 1985). OPH for myocardial contractility was, therefore, judged by the recovery of V_{max} and dP/dt_{max} during reperfusion.

Previously, we observed that decreasing temperature to 28°C and below would injure the cardiac contractility *in vivo* (Ning *et al.*, 2008). Therefore, an OPH should be 29°C or higher. A core temperature $\leq 35^\circ\text{C}$ is considered as hypothermia (Papile, 2005; Gibson and Andrews, 2013), and the OPH should also be lower than 35°C. Previously, we demonstrated that an OPH exists in the isolated rabbit hearts (Xu *et al.*, 1996; Ning *et al.*, 1998c, 1999). The good recovery was at 29–30.5°C, which was significantly different from the poor recovery at 34–35°C ($p < 0.01$). In this study, we progressively reduced core temperature to the target levels for 20 minutes before arrest, 60 minutes during arrest; rewarmed to 37°C within 20 minutes. Then, the pump was turned off. Off-pump recording was continued for 40 minutes (60 minutes after arrest). The optimal temperature for preservation of myocardial contractility, dP/dt_{max} , and V_{max} was determined as the inflection point on the plot of good recovery curves (Figs. 1 and 2).

To determine nonprotective temperature. In the pilot study, we have tested the arrest temperature range from 35°C to 29°C. The preliminary data showed that above 34°C, there was no significant protection. Temperatures around 29–30.5°C showed the best functional recovery. The nonprotective temperature for poor recovery of the dP/dt_{max} and V_{max} was determined as the inflection point conjugated to the poor recovery plateau (Figs. 1 and 2).

Statistical analysis

The reported values are means $\pm$ standard error in the text, table, and figures. The S-PLUS Version 6.2 Program (In-

FIG. 1. The OPH for V_{max} recovery (mean $\pm$ SE). The recovery curve of V_{max} shows a sigmoid pattern in the 17 hearts *in vivo* at 20 minutes of “off-pump” (at 60 minutes of reperfusion). There are two plateaus: One is at 29–30.5°C range, where the V_{max} recovered completely ($113.6 \pm 5.5\%$ of baseline). A point of inflection exists at the edge (around at 30.5–31°C) of high recovery plateau followed by a steep slope that merges into a poor recovery plateau at 34–35°C ($67.2 \pm 5.2\%$ of baseline, $p < 0.05$ compared with 29–30.5°C arrest). The 32–32.5°C arrest shows some improvement ($82.4 \pm 3.7\%$ of baseline) but is poorer than 29–30.5°C arrest ($p < 0.01$); its position is in the middle area of the slope. The OPH for the V_{max} recovery should be at the right side of the inflection point, which is around 30.5–31°C. The numbers on the X axis are the heart temperature 20 minutes before and 60 minutes during arrested period. SE, standard error; V_{max} , velocity of myocardial contraction; on the Y axis (% of baseline), the function recovery at 20 minutes of “off-pump” (% of baseline); OPH, optimal protective hypothermia.

sightful Corp., 2003) was used for statistical analysis. Data were evaluated with repeated-measure analyses of variance within groups and single factor analysis of variance across groups. When significant F values were obtained, individual group means were tested for differences. The criterion for significance was $p < 0.05$ for all comparisons (Ning *et al.*, 1998c, 2007a, 2007b, 2008).

Results

The OPH was determined by sigmoid curves of V_{max} and dP/dt_{max}

The treatment with a temperature lower than normal body temperature to reach $\leq 35^\circ\text{C}$ on the core temperature is considered as hypothermia (Swinhar, 2005; Gibson and Andrews, 2013). The protective hypothermia would be below 35°C and above the cooling injury threshold (29°C) (Ning *et al.*, 2008). An OPH should show the best recovery after cardiac arrest. The 17 hypothermic arrested hearts *in vivo* at 20 minutes of off-pump during reperfusion showed that the recovery curves of V_{max} and dP/dt_{max} exhibited sigmoid pattern: a good recovery plateau at 29–30.5°C arrested hearts, which recovered to baseline level (BL) ($V_{max} = 118.4 \pm 3.9\%$ of BL, $LV\ dP/dt_{max} = 120.7 \pm 3.1\%$ of BL, $n = 6$). A point of inflection existed at the edge of high recovery plateau (about 30.5–31°C) followed by a steep slope until merging

FIG. 2. The OPH for $dP/dt_{\max}$ recovery (mean $\pm$ SE). The recovery curve of $dP/dt_{\max}$ shows a sigmoid pattern in the 17 hearts *in vivo* at 20 minutes of “off-pump” (at 60 minutes of reperfusion). There are two plateaus: One is at 29–30.5°C arrest; another is at 34–35°C arrest. A steep slope is between the two plateaus. A point of inflection exists at the edge of the high recovery plateau. The OPH for the $dP/dt_{\max}$ recovery should be at the right side of the inflection point, which is around 30.5–31°C. The numbers on the X axis are the heart temperature 20 minutes before and 60 minutes during arrested period. $dP/dt_{\max}$, maximum of the positive first derivative of left ventricular pressure; on the Y axis (% of baseline), the function recovery at 20 minutes of “off-pump” (% of baseline).

into a poor recovery plateau at 34–35°C ($V_{\max} = 60.2\% \pm 2.8\%$ of BL, LV $dP/dt_{\max} = 28.0\% \pm 5.9\%$ of BL, $n = 6$, $p < 0.05$ vs. good recovery plateau). The values are similar to the normothermia arrested hearts ($V_{\max} = 55.9\% \pm 7.8\%$ of BL, LV $dP/dt_{\max} = 25.4\% \pm 4.9\%$ of BL, $n = 4$, $p > 0.05$ vs. 34–35°C treatment). The point of inflection on the high recovery plateau represents an OPH around 30.5–31°C (Figs. 1 and 2). The 32–32.5°C arrested hearts showed some recovery ($V_{\max} = 84.4\% \pm 3.5\%$ of BL, LV $dP/dt_{\max} = 78.8\% \pm 6.6\%$ of BL, $n = 5$) in the middle area on the slope of the sigmoid curves (Figs. 1 and 2). There was less injury with arrest effect than in the 34–35°C, and the recovery was lower than in the 29–30.5°C arrest ($p < 0.05$). The current results are in accordance with previous observations in the isolated rabbit hearts (Ning *et al.*, 1998a, 2007b).

Cardiac function recovery in hypothermia arrested hearts

The hemodynamic indices of hypothermia arrested hearts are listed in Table 1. Figure 3 shows the LV DP full recovery to baseline levels in the 29–30.5°C arrested hearts. The recovery remains stable during off-pump after rewarming (DP = $105.8\% \pm 3.5\%$ of BL in the 10–20 minutes off-pump period). The DP in 32–32.5°C arrested hearts recover almost as well as the 29–30.5°C arrested hearts (DP = $96.8\% \pm 6.9\%$ of BL), while the 34–35°C arrested hearts have a very poor recovery (DP = $42.5\% \pm 6.3\%$ of BL).

The CP (Fig. 4) recovers above baseline levels and remains stable during off-pump after rewarming in the 29–30.5°C (CP = $139.5\% \pm 3.6\%$ of BL in the 10–20 minutes off-pump period) and in the 32–32.5°C arrested hearts (CP = 119.0%

TABLE 1. HEMODYNAMICS (MEAN $\pm$ STANDARD ERROR)

	Baseline	REP 30 minutes	REP 40 minutes
DP (mmHg)			
C	95.3 $\pm$ 8.9	49.2 $\pm$ 7.0	45.8 $\pm$ 9.8
H	75.3 $\pm$ 2.1	77.5 $\pm$ 2.0 ^a	77.7 $\pm$ 1.8 ^a
M	83.1 $\pm$ 3.4	77.1 $\pm$ 3.7 ^a	77.8 $\pm$ 3.8 ^a
$dP/dt_{\max}$ (mmHg/s)			
C	2056 $\pm$ 216	1045 $\pm$ 200	910 $\pm$ 271
H	2026 $\pm$ 105	2309 $\pm$ 131 ^a	2401 $\pm$ 116 ^a
M	2018 $\pm$ 217	1875 $\pm$ 215 ^a	1960 $\pm$ 261 ^a
– $dP/dt_{\max}$ (mmHg/s)			
C	–1073 $\pm$ 134	–445 $\pm$ 134	–473 $\pm$ 129
H	–998 $\pm$ 63	–1137 $\pm$ 79 ^a	–1137 $\pm$ 73 ^a
M	–1667 $\pm$ 310	–1253 $\pm$ 144 ^a	–1272 $\pm$ 135 ^a
HR (beats/min)			
C	136.1 $\pm$ 7.9	78.0 $\pm$ 8.0	69.8 $\pm$ 11.1
H	136.3 $\pm$ 4.2	151.9 $\pm$ 12.8 ^a	136.1 $\pm$ 5.2 ^a
M	146.4 $\pm$ 7.8	136.7 $\pm$ 12.3 ^a	134.8 $\pm$ 12.7 ^a
CO _{peak} (L/min)			
C	4.68 $\pm$ 0.52	2.75 $\pm$ 0.61	2.29 $\pm$ 0.46
H	5.26 $\pm$ 0.56	6.78 $\pm$ 0.71 ^a	7.24 $\pm$ 0.81 ^a
M	6.58 $\pm$ 0.81	8.85 $\pm$ 1.52 ^a	8.98 $\pm$ 1.49 ^a
Power _{max} (W)			
C	1.055 $\pm$ 0.228	0.347 $\pm$ 0.079	0.296 $\pm$ 0.098
H	0.869 $\pm$ 0.092	1.555 $\pm$ 0.115 ^a	1.244 $\pm$ 0.141 ^a
M	1.243 $\pm$ 0.176	1.547 $\pm$ 0.308 ^a	1.568 $\pm$ 0.300 ^a
$V_{\max}$ (units/s)			
C	21.84 $\pm$ 2.44	15.08 $\pm$ 3.73	13.24 $\pm$ 1.66
H	25.76 $\pm$ 1.77	28.56 $\pm$ 2.39 ^a	30.25 $\pm$ 1.97 ^a
M	23.22 $\pm$ 2.29	21.84 $\pm$ 2.53	19.69 $\pm$ 2.37 ^a

The hemodynamic indices were determined in the hearts at baseline, 10 and 20 minutes of off-pump (30 and 40 minutes of reperfusion) during rewarming reperfusion as described in the “Materials and Methods” section.

^a $p < 0.05$, compared with C.

C, at 34–35°C hypothermia treatment of combining before and during arrest ($n = 6$); H, hypothermia treatment of combining before and during arrest at 29–30.5°C ($n = 6$); M, hypothermia treatment of combining before and during arrest at 32–32.5°C ($n = 5$); CO_{peak}, peak value of cardiac output; DP, developed pressure; $\pm dP/dt_{\max}$, maximum of the positive or negative first derivative of left ventricular pressure; HR, heart rate; Power_{max}, maximum of cardiac power; $V_{\max}$, velocity of myocardial contraction.

$\pm 14.6\%$ of BL). The 34–35°C arrested hearts have very poor recovery (CP = $22.6\% \pm 7.0\%$ of BL).

The HR (Fig. 5) recovers to baseline level and remains stable during off-pump after rewarming in the 29–30.5°C arrest (HR = $106.2\% \pm 3.6\%$ of BL in the 10–20 minutes off-pump period). The 32–32.5°C arrest has a moderate recovery (HR = $87.0\% \pm 6.2\%$ of BL), while 34–35°C arrest shows a poor recovery (HR = $63.9\% \pm 6.9\%$ of BL).

Cardioplegic arrest at 34–35°C ended in serious stunning (significantly lowering of DP, CO, and HR) after rewarming. However, at 29–30.5°C, the arrest ended in good functional recovery, suggesting that 29–30.5°C hypothermia plays an essential protective role in the cardiac function recovery. $V_{\max}$ and LV $dP/dt_{\max}$ represent myocardial contractility (inotropic effect) and HR represents chronotropic effect, respectively (Ning and Zhou, 1979). These parameters are not well recovered in 32–32.5°C arrest, suggesting that this temperature only partially protects cardiac pump. However,

FIG. 3. Left ventricular DP after hypothermic arrest (mean $\pm$ SE). The numbers on the X axis are the time in the 10–20 minutes of “off-pump.” The DP recovers completely after 29–30.5°C arrest (H, $n=6$). The 34–35°C arrest (C, $n=6$) shows a poor recovery, while the 32–32.5°C arrest (M, $n=5$) shows some improvement. $**p < 0.01$, H and M compared with C. On the Y axis (% of baseline), the function DP recovery of the arrested hearts (% of baseline); DP, developed pressure.

DP and Power are well recovered in 32–32.5°C arrest, implying a good regulation of peripheral vascular system. Since LVEDP was controlled by CPB, the heterometric autoregulation (Starling effect) (Ning *et al.*, 1985) was not involved in this cardiac arrested preparation. In addition, a risk of clinically significant arrhythmias occurs below 30°C (Polderman and Herold, 2009). The 30.5–31°C would be safe as an optimal hypothermia.

Discussion

Hypothermia treatment has been used for 60 years as a protective method to avoid hypoxic/ischemic injury (Ning and Chen, 2006). While some documents reported significant

FIG. 4. Cardiac power (Power) after optimal hypothermic arrest (mean $\pm$ SE). The numbers on the X axis are the time in the 10–20 minutes of “off-pump.” The power recovers completely after 29–30.5°C arrest (H, $n=6$). The 34–35°C arrest (C, $n=6$) shows a poor recovery, while the 32–32.5°C arrest (M, $n=5$) shows improvement. $**p < 0.01$, H and M compared with C. On the Y axis (% of baseline), the function power recovery of the arrested hearts (% of baseline).

FIG. 5. HR after optimal hypothermic arrest (mean $\pm$ SE). The numbers on the X axis are the time in the 10–20 minutes of “off-pump.” HR recovers completely after 29–30.5°C arrest (H, $n=6$). The 34–35°C arrest (C, $n=6$) shows a poor recovery, while the 32–32.5°C arrest (M, $n=5$) shows some improvement. $*p < 0.05$, H compared with C; $**p < 0.05$, H and M compared with C. On the Y axis (% of baseline), the function recovery of the arrested hearts (% of baseline); HR, heart rate.

functional improvement (Testori *et al.*, 2013) with hypothermic treatment, others reported no benefit for the heart (Dae *et al.*, 2002; Nielsen *et al.*, 2013). Myocardial cooling with infusion of 15°C in the un-arrested state resulted in significant myocardial damage (Rebeyka *et al.*, 1990; Perlman *et al.*, 2012). Preischemic hypothermia at 17°C had a deleterious effect on postischemic recovery of hearts in neonatal lambs (Aoki *et al.*, 1994) that might be related to Ca^{2+} increase (Polderman and Herold, 2009). Some researchers recommended the avoidance of rapid myocardial cooling on bypass. However, therapeutic hypothermia has been widely used. Since the 1990s, mild hypothermic protection has gotten a new look (Swinhar, 2005; Choi *et al.*, 2013; Søreide *et al.*, 2013; Bernard, 2014), as patients are protected to tolerate systemic hypoxic injury during incident rescue (Bernard, 2004) and to resist ischemic injury during surgery (Hollenbeck *et al.*, 2012). The optimal temperature, method, onset, duration, rewarming rate, and therapeutic window remain unclear (Koreny *et al.*, 2009; Erb *et al.*, 2012; Leshnower *et al.*, 2012; Miculescu *et al.*, 2013).

The clinical observations reported different results with different treated conditions. Different effects were also seen in adult animals (Ning *et al.*, 1998b) and neonates (Aoki *et al.*, 1994). The lack of sufficient data with a rigorous scientific approach for optimal hypothermia studies is a major factor that induces different results. The ILCOR has published guidelines for the management of cardiac arrest (International Liaison Committee on Resuscitation, 2006; Gibson and Andrews, 2013). A recommendation for targeted temperature management (TTM) was 32–34°C for adult cardiac arrest and 32.5–35.5°C for newborns (Perlman *et al.*, 2012). Some reports indicated that hypothermia “dose” ($\leq 34^\circ\text{C}$ and $\leq 35^\circ\text{C}$) was not associated with survival or good outcome (Huynh *et al.*, 2013).

Severe cardiac hypoxia is responsible for significant mortality in an emergency setting: Each year, about 1 in 1500 adults unexpectedly die due to cardiac arrest; many lives are lost due to cardiac incidents before the patient arrives at the

hospital for treatment (Kern, 2012) and less than 5% survive until hospital discharge (Bernard, 2004). Many survivors may not achieve total recovery. We need to confirm an OPH and TTM by using experimental optimal hypothermia as a base in an effective approach. This study aimed at exploring data to support the OPH and TTM.

It has long been thought that reducing metabolic rate results in less energy consumption with improvement in cardiac protection (Ning *et al.*, 1998a, 1998c; Carey *et al.*, 2003; Tissier *et al.*, 2013). When myocardial temperature decreases from 37°C to 31°C, the myocardial oxygen consumption is reduced from 3.9 ± 0.3 to 2.5 ± 0.2 $\mu\text{mol}/\text{min}/\text{g}$, respectively (Ning *et al.*, 2008). Energy demand significantly increases at 34°C and above, making the organ more susceptible to injury from hypoxia/ischemia. Some scholars infer that a mathematical function exists between temperature and protection as the relationship between temperature and a chemical reaction. However, in this study, the cardiac function recovery showed a sigmoidal curve with different arrested temperature treatments (Figs. 1 and 2). The curve at 29–30.5°C forms a plateau that is significantly different from another plateau at 34–35°C ($p < 0.01$). At the right side of the high recovery plateau, a point of inflection exists (around 30.5–31°C) that would be considered as an OPH. From the point of inflection, the curves decline, suggesting a trigger-like effect, which is threshold phenomena. The point around 30.5–31°C is similar to the protective temperature threshold in the isolated rabbit hearts (Ning *et al.*, 1998b, 1998c, 2002). Accompanied with marked functional improvement, histological evidence showed that the hypothermia-treated hearts had less swelling and damage to the myocardium, as well as less collagen in the connective tissue (Ning *et al.*, 2007b).

In addition, an important datum to support trigger-like effect is the cross-adaptation reaction. Hypothermia as a stressor can resist hypoxic stressor-induced injury. Signaling and receptor pathways are involved in the adaptive reactions. For example, some reactions are related to necrosis types of cell death in myocardium. Prevention of ATP loss (Swinhar, 2005) and of mitochondrial dysfunction is a protective mechanism of hypothermia. The nuclear encoded mitochondrial protein, beta subunit F_1 -ATPase (βF_1 -ATPase), and adenine nucleotide translocase-isoform-1 (ANT_1) decrease during ischemia (Ning *et al.*, 1998c, 1999, 2002, 2007b) or hypoxia (Ning *et al.*, 2003), and are preserved by 30°C hypothermia as well as accompanied with higher ATP levels (Ning *et al.*, 1998b, 1998c, 1999). Hypothermia reduces both apoptosis and necrosis types of cell death in myocardium (The Hypothermia After Cardiac Arrest Study Group, 2002; Ning *et al.*, 2007b). Succinodehydrogenase (SD) codes for a respiratory chain enzyme that is important in the aerobic metabolism and was stimulated to overexpress mRNA as a feedback response to ischemia. It is diminished by 30°C hypothermia (Ning, 2002, 2007b). Hearts adapted to 30°C hypothermia show a decreased p53 (Ning, 2003, 2007b), which is relevant to apoptosis. Hypoxia-induced factor-1 α ($\text{HIF}1\alpha$) codes for a protein that increases in 30°C hypothermia-treated hearts (Ning *et al.*, 1998c, 2002) with a characteristic adaptive functional response. The glucose regulated protein 94 (Grp94) can be induced by a change in temperature and hypoxia (Ning *et al.*, 2007a). Cytochrome P-450 might be involved in hypothermia protection (Ning *et al.*, 2002). Thirty degree celsius induces HSP70-1 that plays an essential role in

tissue protection (Ning *et al.*, 1999, 2002). Heme oxygenase 1 ($\text{HMOX}1$), peroxisomal proliferator-activated receptor β ($\text{PPAR}\beta$), and protein kinase B (Akt-1) are activated in rabbit hearts by 30°C hypothermia (Ning *et al.*, 2007b). Hypothermia also increases ($\text{AKT}1$) gene function in the mouse hearts. (Beiser *et al.*, 2010), increases $\text{TNF}\alpha$ and $\text{IL}6$ mRNA in the rabbit hearts (Ning *et al.*, 2007b), improves functional recovery in the rat hearts (Stadelmann *et al.*, 2013), and decreases reactive oxygen species (Tissier *et al.*, 2013).

This study and the data collected from isolated rabbit hearts (Ning *et al.*, 2007b), rat hearts (Hyyti *et al.*, 2006; Holzer and Behringer, 2008), murine hearts (Beiser *et al.*, 2010), and pig hearts *in vivo* (Ning *et al.*, 2008) indicate that around 30°C is the optimal hypothermia to protect arrested hearts. Although the “mild hypothermia” clinically used is in the range 32–35°C (Kern, 2012), higher than 34°C seems to be less protective (Don *et al.*, 2009). Patients with acute myocardial infarction were treated by endovascular cooling to $33.2^\circ\text{C} \pm 0.9^\circ\text{C}$ for 3 hours during reperfusion and showed no significant decrease in infarct size (Flores *et al.*, 2004). These results might be explained as that temperature $\geq 34^\circ\text{C}$ treatment had no significant benefit and 32–32.5°C had some benefit effect; if 32–35°C treatments were put together as one group, the statistical analysis might not show significant benefits. A report indicated that in an upper body surgery for coronary artery disease treated with 29°C hypothermia, the patient had a good postoperative outcome (Flores *et al.*, 2004). On the contrary, the treatment with 33°C versus 36°C hypothermia did not improve patient recovery (Nielsen *et al.*, 2013). The Seattle Harborview Medical Center has reported that in treating cardiac arrest patients with hypothermia (32°C central catheter) resuscitation showed beneficial effects (Don *et al.*, 2009). Recently, a patient suffered from a sudden heart stop for more than 6 minutes. He was placed in 24-hour cooling protocol postarrest in the Harborview Medical Center. Noninvasive surface cooling pads (Arctic Sun 2000; Medivance) (Kupchik, 2009) with circulating water were placed on his skin covering the body surface area. A right subclavian central catheter was used to set the core temperature first to 32°C, followed by treatment at 33–35°C for 24 hours. Three days later, the patient’s cardiovascular status was dramatically improved (Ning *et al.*, 2010). After 17 months, the cardiologic follow up showed that the patient was similar to “a resuscitated sudden death survivor, who has fully recovered.” He is continuously working on his cardiac research.

In addition, the risk of clinically significant arrhythmias occurs below 30°C (Polderman and Herold, 2009); therefore, optimal hypothermia at around 31°C (Figs. 1 and 2) would not increase arrhythmia problem.

Conclusion

In this study we determined the optimal protective hypothermia (OPH) in pigs. The results are concordant with previous data from murine, rat and rabbit hearts. The OPH at around 31°C showed the most beneficial effect in protecting the arrested heart. These data suggest that the initial core temperature should be set to 31°C in treating acute cardiac arrest patients.

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