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PRECLINICAL RESEARCH

Selective Autoretroperfusion Provides Substantial Cardioprotection in Swine

Incremental Improvements With Mild Hypothermia

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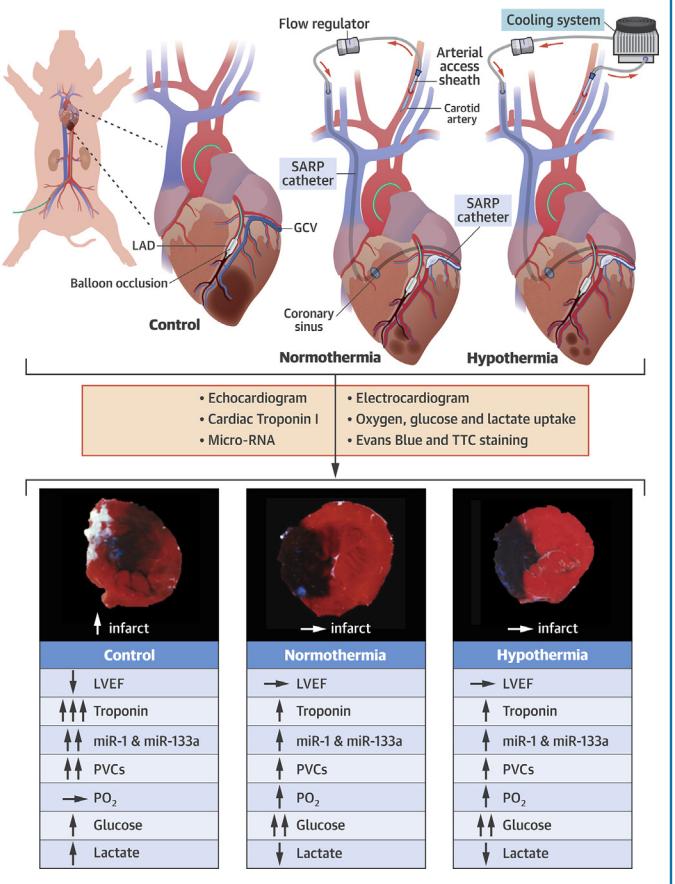
VISUAL ABSTRACT



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HIGHLIGHTS

- **SARP of coronary veins alone or in combination with focal MH-SARP provided cardioprotection following occlusion of the left anterior descending artery.**
- **Significant reduction in infarct size was achieved with MH-SARP and SARP with preservation of myocardial function and cell integrity.**
- **MH-SARP or SARP may provide a clinically relevant percutaneous short-term option of cardiac support to high-risk patients undergoing percutaneous coronary intervention.**

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ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

cTnI = cardiac troponin I

EF = ejection fraction

GCV = great cardiac vein

IABP = intra-aortic balloon pump

LAD = left anterior descending coronary artery

LV = left ventricle/ventricular

MH = mild hypothermia

miR = microRNA

PCI = percutaneous coronary intervention

PO_2 = partial pressure of oxygen

SARP = selective autoretroperfusion

STEMI = ST-segment elevation myocardial infarction

SUMMARY

Mild hypothermia (MH) and retroperfusion are 2 techniques proposed to reduce infarct size due to myocardial infarction. The authors evaluated the effects of focal MH combined with selective coronary venous autoretroperfusion (SARP) as an acute cardioprotective modality before percutaneous coronary intervention (PCI) in a swine model of left ventricular myocardial infarction. Significant reduction in infarct size with preservation of cardiac function and cardiomyocyte viability were achieved. The authors propose that SARP alone or in combination with MH may provide a clinically relevant percutaneous short-term option of cardiac support to high-risk patients undergoing PCI. (J Am Coll Cardiol Basic Trans Science 2020;5:267–78)

Coronary heart disease continues to be the leading cause of morbidity and mortality in the United States and worldwide (1), with an estimated 8 million deaths in 2016 (31% of all global deaths) according to the World Health Organization (2). Acute coronary syndrome, which includes the diagnoses of myocardial infarction—ST-segment elevation myocardial infarction (STEMI) or non-STEMI—and unstable angina, places a heavy clinical and financial burden on the health care system, STEMI being the most severe form of acute coronary syndrome, affecting more than half a million Americans each year (1). These conditions usually present themselves with a high incidence of cardiogenic shock (3), which places these patients in a group at high surgical risk for revascularization,

SEE PAGE 279

along with a group of patients with multiple comorbidities such as stroke, diabetes, heart failure with low ejection fraction (EF), cardiac arrhythmias, and advanced age (4). Temporary percutaneous left ventricular (LV) assist devices such as the Impella

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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(Abiomed, Danvers, Massachusetts) or the intra-aortic balloon pump (IABP) (Johns Hopkins, Baltimore, Maryland) have been said to offer hemodynamic support (via improved perfusion) to this group of patients, allowing the interventional cardiologists to undertake these high-risk procedures (5). A recent systematic review, however, of 20 studies (4 small randomized controlled trials and 16 observational studies) evaluating the safety and efficacy of the Impella and the IABP for high-risk percutaneous coronary interventions (PCIs) concluded that, although the Impella appeared to offer better hemodynamic support and procedural characteristics than the IABP, the number of available studies supporting the effectiveness of either device was small, and all observational studies had a high or critical risk of bias (4). Furthermore, it now appears that the IABP for cardiogenic shock offers no benefit, and it is no better than placebo (6). Whichever the case may be, this type of cardioprotection is ineffective in patients with severely occluded coronary arteries.

Mild hypothermia (MH), defined as a temperature of 32°C to 35.9°C, has been reported to provide cardioprotection and decrease in infarct size following a STEMI by reducing myocardial metabolic demand, free radical production, and platelet aggregation (7). Clinical translation of these cardioprotective results, however, has not been successful because of an inability to locally cool the ischemic region before PCI (8,9). Unlike the obstructed coronary arterial system, the coronary venous system remains unobstructed and thus has great potential for therapy delivery (retrograde delivery of arterial blood flow—retroperfusion—with and without MH). To date, therapeutic retroperfusion has not been adopted clinically (although the concept was introduced more than a century ago) because complicated equipment is required to regulate perfusion to prevent damage to the entire coronary venous system when exposed to arterial pressures (9).

The purpose of the present study was to evaluate the effects of selective autoretroperfusion (SARP) and MH-SARP on offering temporary myocardial support (i.e., reduced demand through hypothermia and increased perfusion through SARP) when applied following coronary artery occlusion before PCI. A novel SARP catheter (9,10) regulates the pressure reaching the venous system to locally deliver either normothermic or cooled arterial blood to the ischemic region of the myocardium. The proposed strategy conferred cardioprotection during coronary occlusion in swine and warrants clinical translation for myocardial preservation in high-risk patients undergoing complex procedures.

METHODS

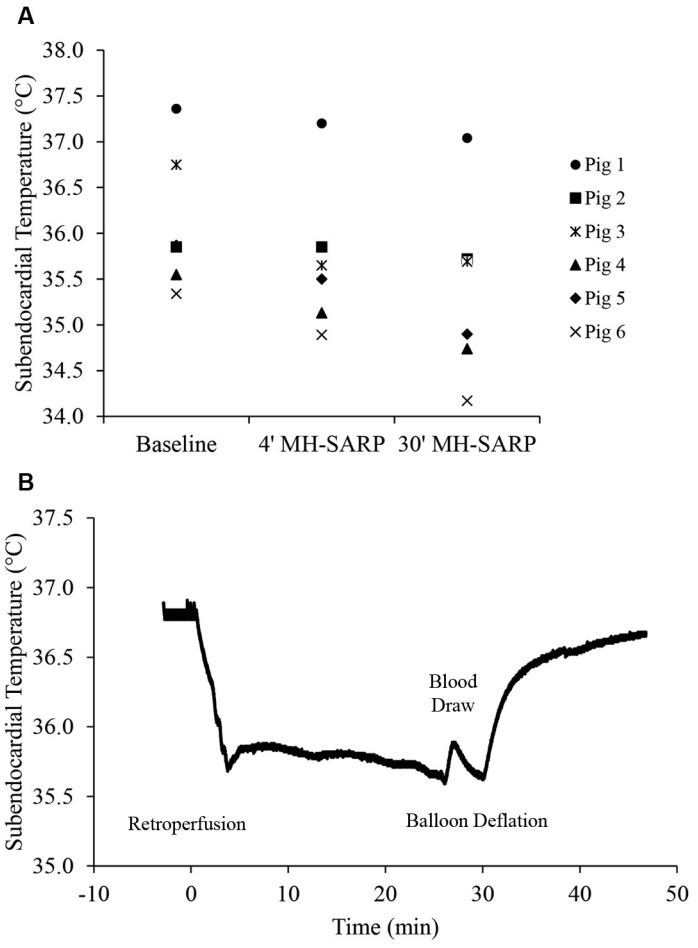
All animal experiments were performed in accordance with national and local ethical guidelines, including the Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act, and an approved California Medical Innovations Institute institutional animal care and use committee protocol regarding the use of animals in research.

ANIMAL PREPARATION. Twenty female Yorkshire domestic swine, with a mean body weight of 49.2 ± 5.4 kg, were divided into 3 groups: MH-SARP (n = 6), normothermia SARP (n = 7), and sham control (n = 7). The pigs were fasted overnight. Sedation was achieved with ketamine, 10 mg/kg intramuscularly, and surgical anesthesia was maintained with isoflurane 1.5% to 2.5%. A 3-mm Maverick over-the-wire balloon catheter (Boston Scientific, Marlborough, Massachusetts) was inserted through the right femoral artery and positioned under fluoroscopic guidance into the left anterior descending (LAD) coronary artery, distal to the second diagonal branch. The temperature of the subendocardium (**Figure 1**) was measured via a sterile custom percutaneous temperature probe consisting of a 5-F radial catheter with an 18-ga needle affixed within the distal tip of the catheter such that 3.5 mm of the needle protruded from the catheter. The SARP catheter was inserted through the right jugular vein, advanced into the coronary sinus, and then positioned in the great cardiac vein (GCV) between the base of the heart and the anterior interventricular sulcus. With all catheters in place, baseline measurements (echocardiography, blood sample collection, arterial pressure, and electrocardiogram recording) were taken before initiation of the procedure. The heart preparation at the end of the study has been described elsewhere (10).

MH-SARP SYSTEM. The system was composed of an arterial access sheath, an extracorporeal Peltier cooling system used in conjunction with a stainless steel heat-transfer heat exchanger, an inline drug delivery port, a flow control mechanism, and the custom delivery SARP catheter. Arterial blood, shunted from the right carotid artery, passed via silicone tubing through the heat exchanger, and was then delivered to the GCV connected to the SARP catheter. The arterial blood was delivered into the GCV using the animal's own pulse pressure (i.e., autoretroperfusion) without the need of synchronized pumps.

In all 3 groups, the LAD artery was occluded for 60 min and then reperfused. In the MH-SARP and

FIGURE 1 Degree of Temperature in the Subendocardium



(A) Subendocardial temperature in the MH-SARP cohort during the experimental procedure. **(B)** Representative experimental tracing obtained from the subendocardial temperature probe showing a regional decrease in temperature when retroperfusion was instituted, and later an increase in temperature when retroperfusion was culminated (balloon deflation). MH = mild hypothermia; SARP = selective autoretroperfusion.

normothermia SARP groups, therapy was initiated following 30 min of LAD artery occlusion and instituted for 30 min while the artery remained occluded. The animals were followed up for 4 weeks.

ECHOCARDIOGRAPHY. Two-dimensional transesophageal and transthoracic echocardiograms were obtained in all animals using an iE33 ultrasound system (Philips, Andover, Massachusetts) for serial measurements of LV function. Long- and short-axes views were obtained during the surgical procedure at 30-min intervals and analyzed offline to determine LV volumes, EF, and wall thickness using QLAB 10.5 (Philips).

BLOOD SAMPLE COLLECTION. Arterial blood, coronary venous blood, central venous blood, and retroperfusion effluent blood samples were collected every 30 min to determine metabolic parameters, including oxygen tension, glucose uptake, lactate uptake, and cardiac troponin I (cTnI) levels, using a handheld i-STAT blood analyzer (Abbott Point of Care, Princeton, New Jersey). MicroRNA-1 (miR-1) and miR-133a levels were measured in plasma. The retroperfusion effluent samples were obtained via the lumen of the LAD balloon catheter while inflated.

STATISTICAL ANALYSIS. All statistical analyses were performed using SigmaStat 3.5 (Systat Software, Point Richmond, California). The data were expressed as mean ± SD. The differences between the various parameters and groups were evaluated using an analysis of variance paradigm, with the between-subjects variable of group (MH-SARP, SARP, and control) and the within-subjects variable (time). In analyses where an interaction between group and time was found to be significant, simple main effects were examined to look at group differences within each time point and to look at time differences within each group. The differences were considered significant at $p < 0.05$. Further, each outcome variable was considered a family, and a Bonferroni correction was used to cap the familywise error rate at 5%.

RESULTS

The hemodynamic parameters in the control, normothermia, and hypothermia groups at baseline, occlusion, retroperfusion, and reperfusion periods are summarized in Table 1. Following the initiation of MH-SARP, the myocardial temperature in the subendocardium (Figure 1A) decreased from 35.9°C ± 0.8°C to 35.1°C ± 1.1°C in <4 min (Figures 1A and 1B). Once MH-SARP treatment was terminated, the subendocardial temperature progressively increased to baseline levels in approximately 15 min (Figure 1B).

The analysis of EF (Figure 2A) showed a significant interaction between group (SARP, MH-SARP, and control) and time (baseline, 30 min, 60 min, and 4 weeks) ($F_{(6,51)} = 56.90$; $p < 0.001$), therefore, simple main effects at each time point were analyzed. There was no significant difference between the 3 groups at baseline ($F_{(2,17)} = 4.83$; p = NS) and 30 min (post-occlusion) ($F_{(2,17)} = 2.64$; p = NS), but there were significant differences in EF at 60 min (post-retroperfusion) ($F_{(2,17)} = 262.86$; $p < 0.001$) and at 4 weeks (end of study) ($F_{(2,17)} = 31.78$; p = NS). At 60 min, Tukey post hoc tests showed that all 3 groups were statistically different from each other (all $p < 0.001$) with the MH-SARP group having a

TABLE 1 Hemodynamic Parameters

	Control	Normothermia	Hypothermia
Baseline			
Systolic BP, mm Hg	81 ± 8	84 ± 8	81 ± 7
Diastolic BP, mm Hg	54 ± 8	58 ± 11	54 ± 8
MAP, mm Hg	66 ± 8	71 ± 11	65 ± 7
Heart rate, beats/min	85 ± 12	86 ± 27	95 ± 32
Pulse pressure, mm Hg	28 ± 3	26 ± 5	28 ± 6
Ischemia			
Systolic BP, mm Hg	62 ± 6***	66 ± 12**	59 ± 4***
Diastolic BP, mm Hg	47 ± 6***	48 ± 10	42 ± 5
MAP, mm Hg	54 ± 6**	56 ± 12*	50 ± 5**
Heart rate, beats/min	85 ± 10	89 ± 26	96 ± 17
Pulse pressure mm Hg	15 ± 2***	18 ± 3**	17 ± 3**
Retroperfusion			
Systolic BP, mm Hg		66 ± 7**	59 ± 5***
Diastolic BP, mm Hg		49 ± 7	44 ± 5
MAP, mm Hg		56 ± 8**	50 ± 5***
Heart rate, beats/min		87 ± 15	99 ± 15
Pulse pressure, mm Hg		17 ± 2	16 ± 2
Reperfusion			
Systolic BP, mm Hg	52 ± 8***	68 ± 7**††	71 ± 6*†††
Diastolic BP, mm Hg	34 ± 10**	46 ± 8†	45 ± 6†
MAP, mm Hg	42 ± 9***	55 ± 8***†	54 ± 6*†
Heart rate, beats/min	78 ± 17	106 ± 37	98 ± 15†
Pulse pressure, mm Hg	17 ± 3***	21 ± 5	26 ± 4††

Values are mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 relative to baseline values. †p < 0.05, ††p < 0.01, †††p < 0.001 relative to control group.
BP = blood pressure; MAP = mean arterial pressure.

significantly higher EF (53.82 ± 0.97%) than the SARP group (48.11 ± 1.61%), and the SARP group having a significantly higher EF than the control group (38.20 ± 1.02%). At 4 weeks, Tukey post hoc tests showed that both the MH-SARP (52.48 ± 3.02%) and SARP groups (50.70 ± 1.31%) had significantly higher EF than the control group (43.19 ± 2.29%; p < 0.001 for both comparisons), and there was no statistically significant difference in EF between the MH-SARP and SARP groups.

The electrocardiogram ST segment demonstrated significant recovery in the degree of segment depression within 10 min following therapy (−0.04 ± 0.19 mV with MH-SARP and −0.06 ± 0.06 mV with SARP vs. −0.14 ± 0.13 mV in the control group; p < 0.05) (Figure 2B). Significant reduction in the number of arrhythmic events (Figure 2C) and absence of QRS distortion post-reperfusion were also observed with SARP (7.3 ± 5.0 arrhythmic events) and MH-SARP (5.5 ± 1.3 arrhythmic events) versus control (27.8 ± 7.9 arrhythmic events; p < 0.01).

A significant interaction between group and time was found for cTnI (Figure 2D) ($F_{(6,45)}$ = 9.83; p < 0.001), therefore, simple main effects at each time point were analyzed. There was no significant difference in cTnI between the 3 groups at baseline ($F_{(2,17)}$ = 1.66; p = NS) and at 2 weeks ($F_{(2,17)}$ = 1.14; p = NS). There was a significant difference, however, in cTnI between the groups at post-reperfusion (90 min [$F_{(2,17)}$ = 9.00; p = 0.002] and 2.5 h [$F_{(2,15)}$ = 10.49; p = 0.001]). At 90 min and 2.5 h, Tukey post hoc tests showed that cTnI was significantly lower in both the MH-SARP (cTnI at 90 min 1.40 ± 0.82 ng/ml; cTnI at 2.5 h 8.13 ± 6.80 ng/ml) and SARP groups (cTnI at 90 min 4.36 ± 3.53 ng/ml; cTnI at 2.5 h 16.80 ± 16.02 ng/ml) compared with the control group (cTnI at 90 min 14.20 ± 9.03 ng/ml; cTnI at 2.5 h 42.64 ± 13.51 ng/ml) (all p < 0.05), and there was no statistically significant difference in cTnI between the MH-SARP and SARP groups.

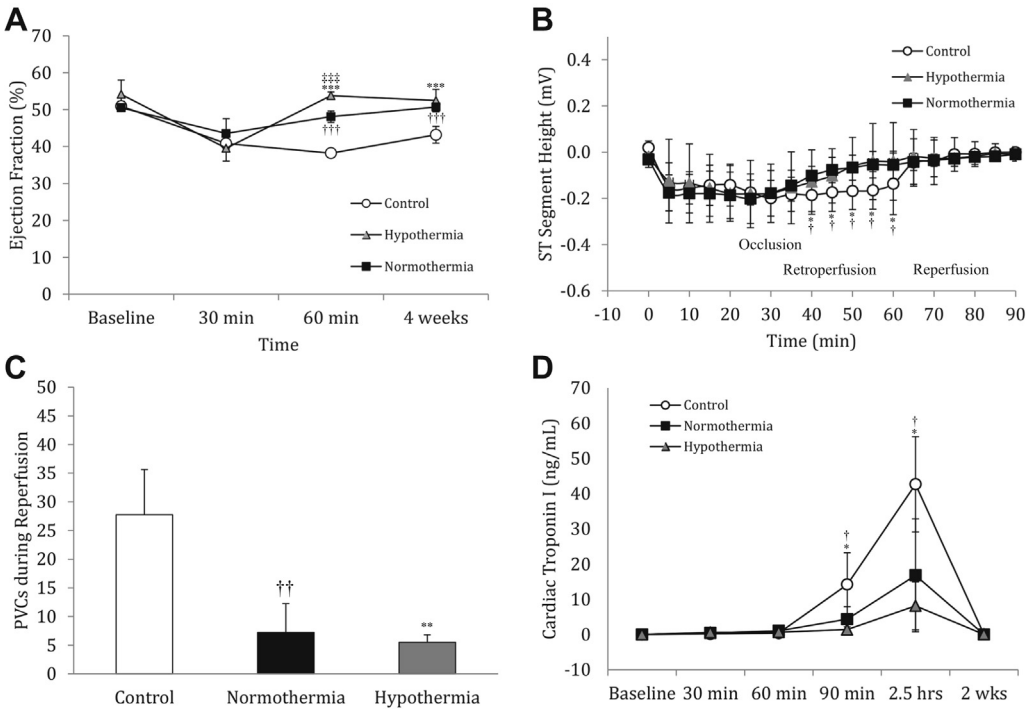
MiR-1 (Figure 3A) and miR-133a (Figure 3B) were quantified using reverse transcription and quantitative polymerase chain reaction. An approximately 7-fold increase (p < 0.05) in miR-1 post-reperfusion was observed in the control group versus baseline. In the SARP and MH-SARP groups, the values increased to 3 and 4 times the baseline values, respectively, but they were not significantly different from baseline. Similarly, miR-133a in the control group also increased to 7 times (p < 0.05) post-reperfusion versus baseline. In the SARP group, miR-133a increased to approximately 7 times at 90 min, although the values were not statistically different from baseline. In the hypothermia group, the values between baseline and 90 min were nearly identical.

Analysis of variance results revealed that percent infarction (relative to the area at risk) (Figure 4A) was significantly different between the 3 groups ($F_{(2,16)}$ = 49.53; p < 0.001). Tukey post hoc tests showed that both MH-SARP infarcted area (1.89 ± 1.43%) and SARP infarcted area (4.74 ± 3.95%) were significantly lower than control infarcted areas (28.11 ± 7.69%; both p < 0.001), and the MH-SARP and SARP groups were not significantly different from each other (p = NS). Figure 4B shows myocardial sections obtained from approximately the same regions in all groups, double-stained with Evans blue and triphenyl tetrazolium chloride. The infarcted area is clearly demarcated in the control group versus SARP and MH-SARP groups.

Figure 5 shows representative histological myocardial sections stained for caspase-3. Caspase-3 expression was elevated in control specimens (Figure 5B) versus SARP (Figure 5C) and MH-SARP (Figure 5D) samples, which approximate healthy viable myocardium (Figure 5A).

Indices of cardiac metabolism with SARP and MH-SARP are shown in Figure 6. A statistically

FIGURE 2 Changes in Ejection Fraction, ST-Segment Height, and Rhythm and Cardiac Troponin Levels



(A) Changes in ejection fraction values at baseline, during occlusion, during therapy with MH-SARP and SARP, and after 4 weeks of reperfusion in all 3 cohorts. **(B)** ST-segment changes in response to the initial ischemic insult (left anterior descending coronary artery occlusion) followed by treatment (SARP and MH-SARP) and reperfusion. **(C)** Arrhythmic events. Frequency of arrhythmic events in the control, normothermia, and hypothermia groups during the reperfusion period. **(D)** Cardiac troponin I (cTnI) levels over time in the control, normothermia, and hypothermia groups. *p < 0.05 between MH-SARP and control groups. †p < 0.05 between SARP and control groups. **p < 0.01 between MH-SARP and control groups. ††p < 0.01 between SARP and control groups. ***p < 0.001 between MH-SARP and control groups. †††p < 0.001 between SARP and control groups. ‡‡‡p < 0.001 between MH-SARP and SARP groups. PVC = premature ventricular contraction; other abbreviations as in Figure 1.

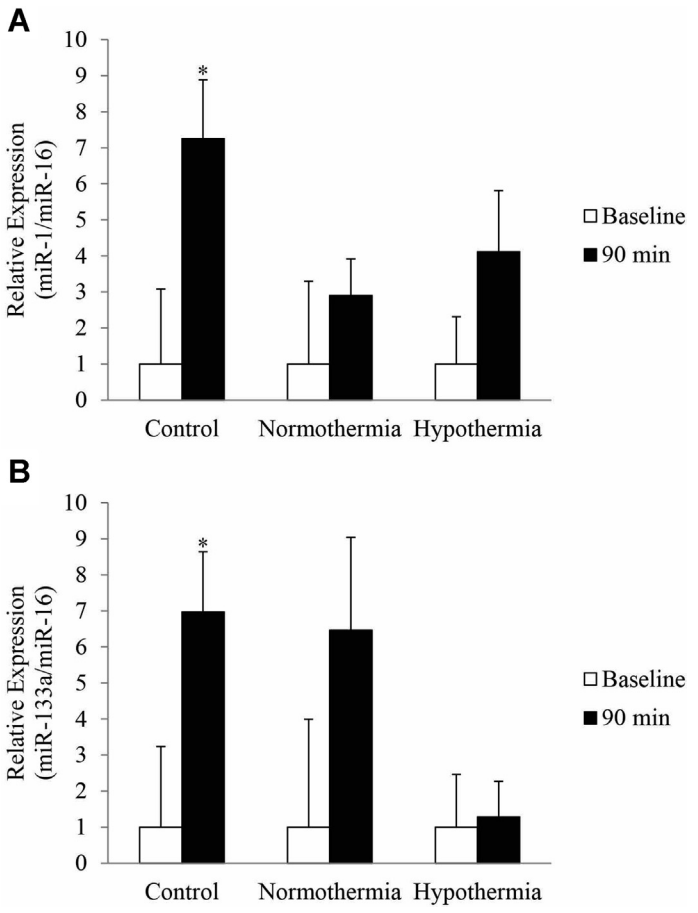
significant group versus time interaction ($F_{(4,34)}$ = 16.68; p < 0.001) was found for partial pressure of oxygen (PO_2) levels in effluent samples (Figure 6A), therefore, simple main effects at each time point were examined. There was no significant difference between the 3 groups at baseline ($F_{(2,17)}$ = 1.44; p = NS). There was a significant difference, however, between the groups at 5 min post-therapy ($F_{(2,17)}$ = 24.60; p < 0.001) and a trend toward a significant difference at 30 min post-therapy ($F_{(2,17)}$ = 4.79; p = 0.022, Bonferroni familywise corrected critical p = 0.017). At 5 min post-therapy, all groups were significantly different from each other (all p < 0.05) with the SARP group showing the highest PO_2 levels (35.19 ± 5.52 mm Hg), followed by MH-SARP (28.32 ± 4.10 mm Hg), with the control group having the lowest PO_2 level (18.01 ± 3.88 mm Hg). Thirty minutes post-therapy, there was no difference in PO_2 levels between the MH-SARP (27.00 ± 1.60 mm Hg) and SARP (28.59 ± 9.00 mm Hg) groups (p > 0.05). In the MH-SARP group, however, PO_2 was significantly higher than in the control group (19.77 ± 2.69 mm Hg; p = 0.025), and there was no significant difference between the SARP and control group (p > 0.05).

Figure 6B shows glucose uptake in effluent samples. A significant group versus time interaction was found ($F_{(4,34)}$ = 18.60; p < 0.001), and therefore, simple main effects at each time point were analyzed. There was no significant difference between the MH-SARP (9.68 ± 9.20 mg/dl), SARP (8.39 ± 4.69 mg/dl), and control (7.50 ± 4.02 mg/dl) groups at baseline ($F_{(2,17)}$ = 0.20; p = NS). Five minutes after SARP, there was a significant overall effect of group ($F_{(2,17)}$ = 44.76; p < 0.001), with both MH-SARP (28.00 ± 3.41 mg/dl) and SARP (24.00 ± 2.11 mg/dl)

being significantly higher than in the control group (11.39 ± 4.19 mg/dl), and no significant difference between the MH-SARP and SARP groups (both $p < 0.001$ by Tukey post hoc test). At 30 min post-therapy, there was also a significant effect of time ($F_{(2,17)} = 9.34$; $p = 0.002$) with the MH-SARP (20.18 ± 4.01 mg/dl) and SARP (18.01 ± 3.28 mg/dl) groups significantly higher than the control group (10.50 ± 5.31 mg/dl) (Tukey post hoc test $p = 0.002$ and $p = 0.012$, respectively), and no significant difference between the MH-SARP and SARP groups.

Figure 6C shows lactate uptake in effluent samples. There was a significant group versus time interaction ($F_{(4,34)} = 22.55$; $p < 0.001$), therefore, simple main effects of group at each time point were examined. At baseline, there were no significant differences between the MH-SARP (0.59 ± 0.37 mmol/l), SARP (0.38 ± 0.21 mmol/l), and the control (0.38 ± 0.41 mmol/l) groups. After 5 min of therapy, the groups were significantly different ($F_{(2,17)} = 20.01$; $p < 0.001$), with lactate uptake significantly lower in MH-SARP (−2.58 ± 1.29 mmol/l) and SARP (−4.40 ± 2.02 mmol/l) groups compared with the control (0.29 ± 0.26 mmol/l) group (Tukey post hoc test $p \leq 0.005$), with no significant difference between MH-SARP and SARP. After 30 min of therapy, the groups were also significantly different ($F_{(2,17)} = 15.28$; $p < 0.001$), with lactate uptake still significantly lower in MH-SARP (−1.44 ± 0.82 mmol/l) and SARP (−1.50 ± 0.60 mmol/l) compared with control (0.62 ± 0.96 mmol/l) (Tukey post hoc test $p \leq 0.001$) with no significant difference between MH-SARP and SARP.

FIGURE 3 Expression of miR-1 and miR-133a



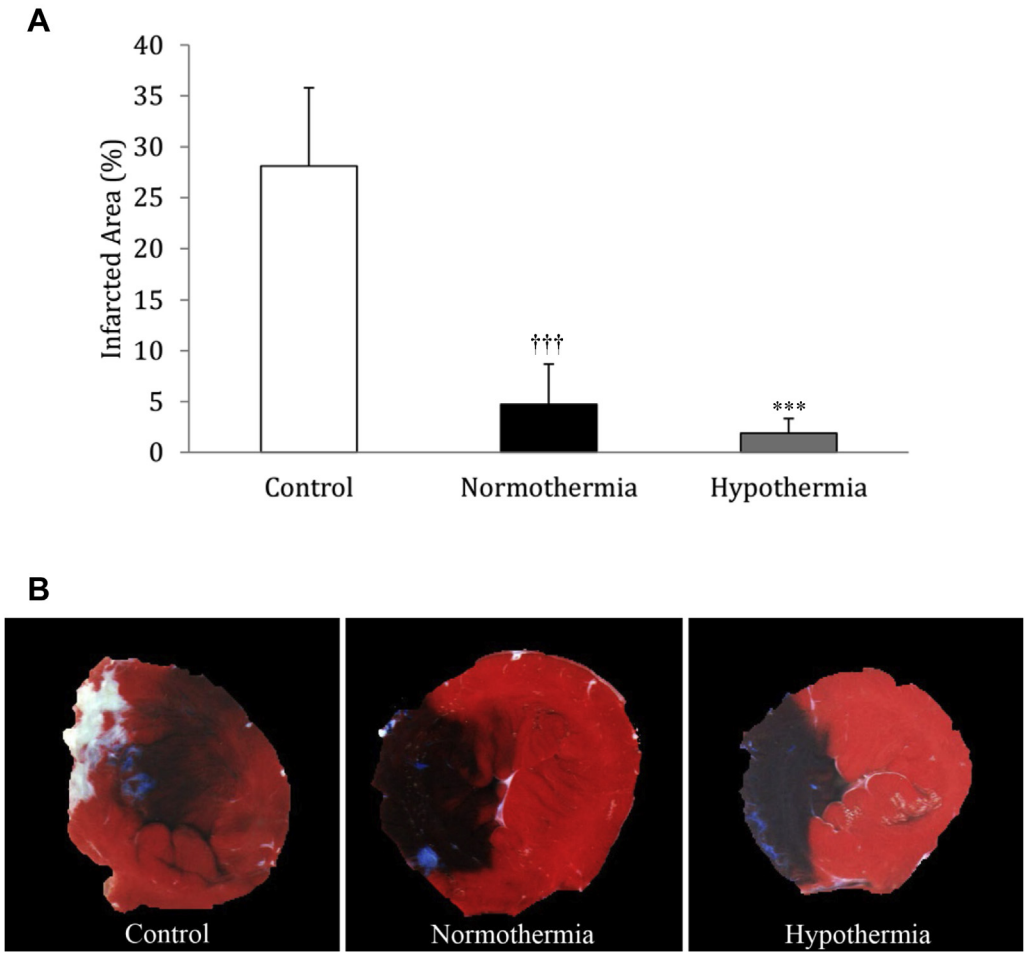
(A) Relative expression of miR-1/miR-16 and **(B)** miR-133a/miR-16 in the control, normothermia, and hypothermia groups at baseline and 90 min. *$p < 0.05$ between baseline and 90 min in the control group. miR = microRNA.

DISCUSSION

We have shown that SARP, alone (10) or in combination with MH, preserves cardiac function and significantly reduces myocardial infarct size up to 98.1% in a swine model of acute myocardial infarction (AMI). Both procedures conferred cardioprotection and were extremely effective in reducing infarct size (98.1 ± 1.4% [93.3% relative to control] for MH-SARP, and 95.3 ± 4.0% [83.2% relative to control] for SARP alone), with concomitant attenuation of markers for myocardial ischemia (cTnI), reperfusion injury (degree of ST-segment depression, miR-1, and miR-133a), and cardiomyocyte injury (oxygen, glucose, and lactate uptake, as well as caspase-3 expression). Most of the benefit, however, was related to SARP alone with little additional benefit to the myocardium when hypothermia was incorporated.

Several animal and clinical studies (11,12) have documented the beneficial effects of hypothermia to minimize infarct size following AMI. Hypothermia has also been reported to decrease cardiomyocyte apoptosis by decreasing activation of caspase-3 (13), an important mediator of apoptosis, which was reduced in the MH-SARP group as shown in **Figure 5D**. Similarly, the beneficial effects of venous retroperfusion for the ischemic myocardium, with (14) and without (10,15) synchronized pumping have been largely investigated. In this study, we evaluated the adjunctive effects of both SARP (without the use of synchronized pumps) to enhance coronary perfusion, and MH to reduce demand as well as oxidative stress, as temporary percutaneous cardiac support before PCI. The reduction in the subendocardial temperature by 1°C did not add much value to the SARP procedure, although a larger decrease in temperature is expected in other regions of the myocardium such as the subepicardium. The small decrease in subendocardial

FIGURE 4 Area of Infarction

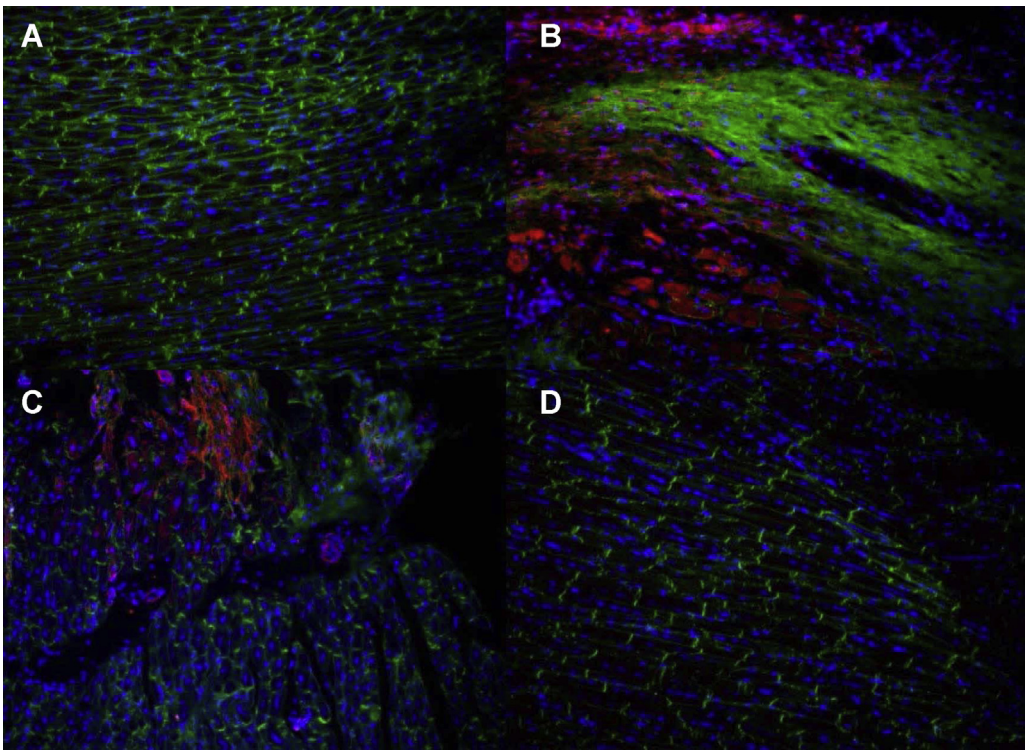


(A) Infarcted area (relative to the area at risk) in the control, normothermia, and hypothermia groups. **(B)** Myocardial sections from control **(left)**, normothermia **(middle)**, and hypothermia **(right)** groups double-stained with Evans blue and triphenyl tetrazolium chloride demarcating the area of infarction. ***$p < 0.001$ between MH-SARP and control groups. †††$p < 0.001$ between SARP and control groups. Abbreviations as in Figure 1.

temperature was an inherent limitation of the technique used. Because the majority of the reduction in infarct size was related to the effects of SARP alone, little additional benefit was available with incorporation of MH regardless of the degree of cooling.

Ventricular assist devices are mechanical pumps that confer temporary cardiac and circulatory support, via improved perfusion, to high-risk patients undergoing elective (16) and emergent (17) PCI. The IABP, the TandemHeart (Cardiac Assist, Pittsburgh, Pennsylvania), and the Impella are the 3 most frequently used LV assist devices in the United States, each with advantages and disadvantages. The IABP, for example, is minimally invasive but does not provide significant benefit to patients with severe cardiogenic shock with a systolic aortic pressure <60 mm Hg (18). The TandemHeart seems to confer better results in this type of patients, but it is more invasive with greater vascular complications (19). The Impella is the latest of the 3 LV assist devices and can increase cardiac output up to 5.0 l/min. The Impella unloads blood from the LV into the aorta, which increases coronary perfusion pressure (20). It also decreases myocardial workload and metabolic consumption, which contributes to a reduction of infarct size in AMI (21). As mentioned in the preceding text, however, these ventricular assist devices offer little protection to patients with obstructed coronary

FIGURE 5 **Assessment of Myocardium by Immunohistochemistry**



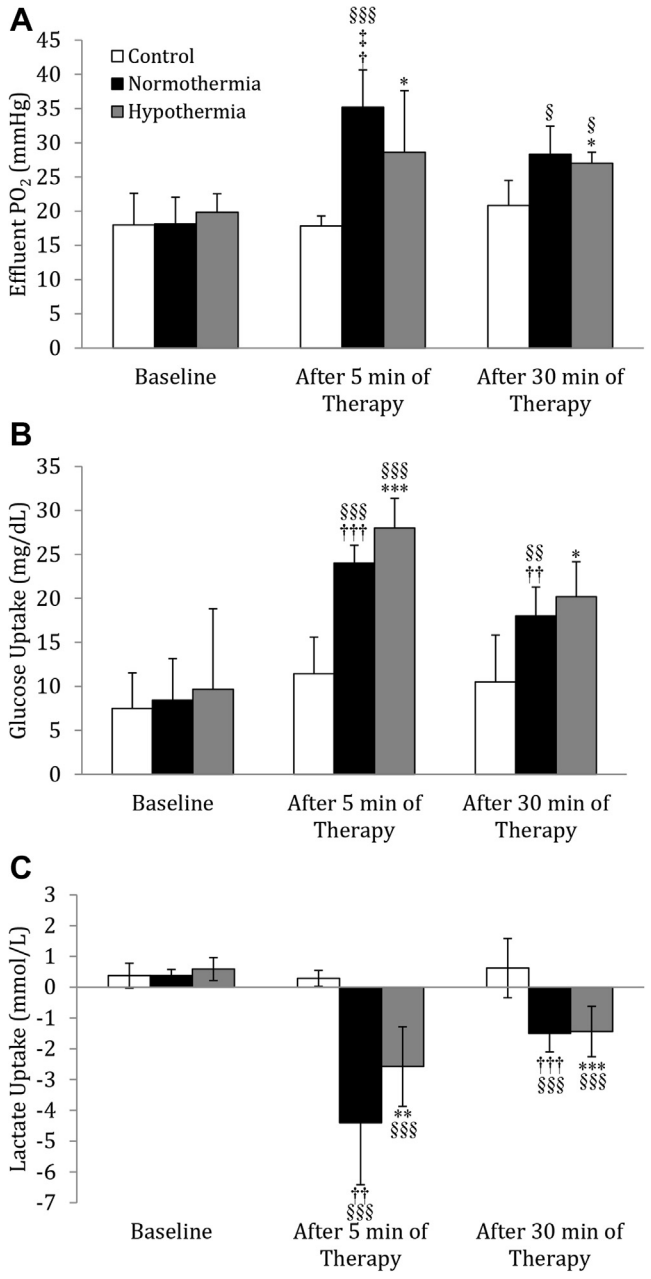
Representative histological samples stained for the reperfusion injury marker caspase-3 **(red)** in healthy viable myocardium **(A)** and control **(B)**, normothermia **(C),** and hypothermia **(D)** groups.

arteries in which SARP or MH-SARP may constitute a better approach.

In the present study, the arterial source was rapidly cooled down using an extracorporeal cooling system and then retroperfused through the coronary veins without the use of pumps. Subendocardial temperature was reduced by ~1°C in <4 min following initiation of therapy. This small reduction in temperature provided an additive (although not statistically different) protective effect to SARP (95.3 ± 4% reduction in infarct size with SARP compared with 98.1 ± 1.4% reduction with MH-SARP). The significant reduction in infarct size observed with MH-SARP (the majority of which was SARP-related) was likely the combined effects of blood supply reaching the ischemic area, removal of adverse metabolites (retroperfusion), and reduction in cellular metabolism (hypothermia), that is, positively affecting the oxygen supply-demand relation. The rapid decrease in subendocardial temperature also supports the effective delivery of blood, which in this case, was confirmed via contrast injection and coronary venogram. Furthermore, measurement of the retroperfusion pressure (38.1 ± 1.6 mm Hg during therapy vs. 20.9 ± 1.7 mm Hg at baseline) in the GCV, distal to the tip of the SARP catheter, indicated that we achieved an ideal pressure (<50 mm Hg), that avoided myocardial edema and hemorrhage (22) without the need of intermittent occlusion of the venous system with external pumps. Moreover, the heart was capable of distributing the blood flow of the ischemic myocardium once blood was delivered through the coronary venous microvessels, facilitating at the same time the washout of toxic byproducts. The superior effects of retroperfusion over intermittent occlusion of the coronary sinus have been reported by Zalewski et al. (23).

MH-SARP and SARP alone significantly reduced the incidence of ventricular arrhythmias during the reperfusion period, which appears to mirror outcomes in patients. The presence of arrhythmias has been attributed to attenuation of conduction, which usually occurs during ischemia and is pre-requisite for re-entry (24). It has been postulated that MH

FIGURE 6 Indices of Cardiac Metabolism With SARP and MH-SARP



Elevation in effluent oxygen **(A)** during retroperfusion supports conversion to anaerobic glycolysis and ischemic metabolism as evidenced by increases in glucose uptake **(B)** and lactate release **(C)** across the treated myocardium. *p < 0.05 between MH-SARP and control groups. †p < 0.05 between SARP and control groups. ‡p < 0.05 between SARP and MH-SARP groups. ***p < 0.001 between MH-SARP and control groups. †††p < 0.001 between SARP and control groups. §p < 0.05 relative to baseline values. §§p < 0.01 relative to baseline values. §§§p < 0.001 relative to baseline values. PO_2 = partial pressure of oxygen; other abbreviations as in Figure 1.

prevents ischemia-induced conduction block and conduction velocity slowing by preserving gap junction coupling as well as sodium channel function (25). It is worth noting that large myocardial temperature gradients can cause severe arrhythmias due to the dispersion of the action potential (26). On the other hand, in the presence of MH, arrhythmias may be completely abolished, which underscores the importance of the degree of hypothermia as an adjunctive therapy in myocardial ischemia. In our study, both MH-SARP and SARP alone significantly reduced the incidence of arrhythmic events post-reperfusion.

Following the initiation of SARP, an increase in effluent PO_2 was observed. This somewhat paradoxical finding suggests a reduced oxygen uptake, which may be the result of cell death or conversion to a glycolytic ischemic metabolism. Support for the latter is provided by marked increase in glucose uptake. These data demonstrate that the onset of anaerobic glycolysis, as evidenced by lactate release across the ischemic bed, may have contributed to the preservation of cell viability. Our main hypothesis is that MH induces a decrease in metabolic demand and hence reduces myocardial cell death post-reperfusion. The results obtained with SARP alone, however, suggest that the primary benefit in this case may be derived by oxygen delivery to the ischemic myocardium and washout of toxic byproducts.

Two biomarkers of myocardial infarction, miR-1 and miR-133a, were strongly up-regulated in plasma from the control group. This up-regulation is likely due to release from the cytoplasm of cardiac cells. On the other hand, nonsignificant up-regulation of miR-1 and miR-133a was found with implementation of SARP or MH-SARP before reperfusion.

STUDY LIMITATIONS. SARP and MH-SARP were applied almost immediately after the initiation of the ischemic period. This schedule of treatment, however, is not clinically relevant because therapy cannot always be applied to patients within 30 min of the beginning of symptoms. Rather than imitations of clinical flow, our intent was to evaluate the technique scientifically. Furthermore, the timing of implementation of SARP or MH-SARP needs to be taken into account before opening the occlusion. Coronary sinus cannulation can be accomplished within 5 min, which is on par with access to the sinus for lead implantation (27), and hence, would not significantly delay the door-to-balloon time. Furthermore, any small delay to the door-to-balloon time is unlikely to affect outcomes (28).

It may be argued that 30 min of LAD occlusion is insufficient to create a meaningful infarct size. Infarct sizes of various magnitudes, however, have been reported in the published reports. Näslund et al. (29), for example, found an infarct size of 45.5 ± 15.7% of the area at risk with 30 min of LAD occlusion, and 78.6 ± 9.3% with 60-min occlusion.

CONCLUSIONS

The data indicate that SARP and MH-SARP preserve myocardial function and cellular integrity, and decrease myocardial infarct size, with the majority of the beneficial effects attributed to SARP alone. The decrease in focal subendocardial temperature by 1°C was not significantly different with respect to the effects of SARP on infarct size reduction, and can be considered negligible. These findings, however, warrant further investigation toward first-in-human translation, which may provide an option as temporary cardiac support in high-risk patients undergoing emergent or selective PCI.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: PCI in high-risk patients carry an elevated mortality rate. SARP alone or in combination with MH preserves cardiac function and myocyte viability, and can be used to provide temporary cardiovascular support to high-risk patients undergoing emergent or elective PCI, as well as in cardiogenic shock patients.

TRANSLATIONAL OUTLOOK: The outcome accomplished in swine with SARP and MH-SARP warrant further investigations toward first-in-human translation.

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