St Thomas' Hospital polarizing blood cardioplegia improves hemodynamic recovery in a porcine model of cardiopulmonary bypass

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ABSTRACT

Objective: Cardiac surgery demands highly effective cardioprotective regimens. We previously demonstrated improved cardioprotection with “polarized” compared with “depolarized” arrest. This study uses a clinically relevant porcine model of cardiopulmonary bypass to compare the efficacy of blood-based St Thomas' Hospital polarizing cardioplegia (STH-Pol-B) with blood-based St Thomas' Hospital hyperkalemic cardioplegia (STH2-B).

Methods: Pigs were monitored and subjected to normothermic cardiopulmonary bypass, cardiac arrest via antegrade cold (4°C) blood cardioplegia (STH2-B, control group: n = 6 or STH-Pol-B, study group: n = 7), and global ischemia (60 minutes) followed by on-pump reperfusion (60 minutes) and subsequent off-pump reperfusion (90 minutes). At termination, tissue samples were taken for analysis of high-energy phosphates, ultrastructure, and microRNAs. The primary endpoint of this study was creatine kinase-muscle/brain release during reperfusion.

Results: Creatine kinase-muscle/brain was comparable in both groups. After pigs were weaned from cardiopulmonary bypass, hemodynamic parameters such as mean arterial pressure ($P = .007$), left ventricular systolic pressure ($P < .001$), external heart work ($P = .012$), stroke volume ($P = .015$), as well as dp/dt_{max} ($P = .027$), were improved with polarizing cardioplegia. Wedge pressure was significantly lower in the study group ($P < .01$). Energy charge was comparable between groups. MicroRNA-708-5p was significantly lower ($P = .019$) and microRNA-122 expression significantly ($P = .046$) greater in STH-Pol-B hearts.

Conclusions: Polarized cardiac arrest offers similar myocardial protection and enhances functional recovery in a porcine model of cardiopulmonary bypass. Differential expression of microRNAs may indicate possible new ischemia–reperfusion markers. These results confirm the noninferiority and potential of polarized versus depolarized arrest. (J Thorac Cardiovasc Surg 2019;158:1543-54)

Polarized is not inferior to depolarized arrest in cardiopulmonary bypass in pigs.

Central Message

In cardiopulmonary bypass in pigs, St Thomas' Hospital polarizing blood cardioplegia shows similar ischemia–reperfusion injury and improves hemodynamic recovery compared with depolarizing cardioplegia.

Perspective

St Thomas' Hospital polarizing blood cardioplegia shows superiority in hemodynamics and non-inferiority in biochemical parameters compared with standard St Thomas' No. 2 blood cardioplegia in a porcine model of cardiopulmonary bypass. Polarized arrest has the potential to improve myocardial protection in cardiac surgery.

See Commentaries on pages 1555 and 1557.

In cardiac surgery, the number of elderly and multimorbid patients has dramatically increased over the last 20 years¹ and is associated with elevated perioperative mortality.²

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Therefore, constant efforts to improve intraoperative myocardial protection are essential to optimize postoperative outcome.

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Abbreviations and Acronyms

AP	= arterial pressure
Ca ²⁺	= calcium
CK-MB	= creatine kinase-muscle/brain
CO	= cardiac output
CPB	= cardiopulmonary bypass
EHW	= external heart work
HMGB1	= high mobility group box 1
HR	= heart rate
IL	= interleukin
LV	= left ventricle
LVP	= left ventricular pressure
miRNA	= microRNA
Na ⁺	= sodium
STH-Pol-B	= new St Thomas' Hospital Polarizing blood cardioplegia
STH2-B	= St Thomas' Hospital blood cardioplegia No. 2
TNF	= tumor necrosis factor
TPM	= tags per million

Scanning this QR code will take you to the article title page to access supplementary information.

Standard (depolarizing) St Thomas' Hospital cardioplegic solution No. 2 (STH2), with a potassium concentration of 16 mmol/L, magnesium of 16 mmol/L, and calcium (Ca²⁺) of 1.2 mmol/L, became one of the most widely used crystalloid cardioplegic solutions worldwide.³ However, hyperkalemic cardioplegic solutions have a narrow extracellular concentration window (potassium between 10 and 30 mmol/L), which can lead to intracellular sodium (Na⁺) accumulation via the noninactivated Na⁺ “window-current,” with subsequent elevation in myocyte Ca²⁺ loading, contracture, and cell death.⁴

A search for a safer means of inducing arrest showed the efficacy of high (mmol/L) concentrations of esmolol.⁵ The arrest characteristics of esmolol act via Ca²⁺-channel and Na⁺-channel blockade.⁶ Recently, Aass and colleagues⁷ demonstrated the efficacy of this new blood-based St Thomas' Hospital polarized cardioplegia (STH-Pol-B) in a cardiopulmonary bypass (CPB) model in pigs. They pointed out the benefits of polarized arrest on the energy status and cardiac index during late ischemia and the early phase of reperfusion. The primary aim of the present study was to describe the effects of low-dose STH-Pol-B on coronary enzyme release (coronary creatine

kinase-muscle/brain [CK-MB], troponin I) during reperfusion. We hypothesized that polarized arrest was noninferior to conventional depolarized arrest. Therefore, in a porcine CPB model, the new blood-based polarizing cardioplegic solution (STH-Pol-B) was compared with standard depolarizing blood-based STH-2, with a view of providing detailed information on cardiac enzyme release, metabolism, hemodynamic functional recovery, and ultrastructure. In addition, our study provides, for the first time, data on the expression of noncoding RNAs, such as myocardial microRNAs (miRNA) in left ventricular tissue samples following CPB. These results may contribute a better understanding of ischemia–reperfusion injury following CPB and provide an indication of whether targeting miRNAs might be a potential novel therapeutic approach to limit myocardial ischemia–reperfusion injury.

METHODS**Animals**

Female pigs (n = 14, Austrian Landrace) were housed at the Center for Biomedical Research, Medical University of Vienna, Austria. Pigs arrived 1 week before experiments for acclimatization and were fed with standard diet twice a day (ssniff GmbH, Soest, Germany) and water ad libitum. The experiments were approved by the Animal Ethics Committee of the Medical University of Vienna (GZ: 66.009/0171-II/3b/2011) and the Austrian Ministry of Science and Technology. All animals received humane care in compliance with the Federation of European Laboratory Animal Science Associations. One experiment had to be stopped during CPB due to an uncontrollable bleeding after aortic cannulation; therefore, 13 pigs were included into the study.

Protocol

Animals were prepared for CPB as described in the [Appendix E1](#). After baseline measurements, CPB was initiated, the aorta crossclamped, and hearts were arrested with antegrade, blood-based cold STH-Pol-B (n = 7) or STH2-B (n = 6). Sixty minutes of ischemia were followed by 60 minutes of on-pump reperfusion (sampling time points: 1, 5, 15, 30, 60 minutes; [Figure 1](#)), and another 90 minutes of off-pump reperfusion (time points: 90, 120, and 150 minutes; [Figure 1](#)). After venous and arterial decannulation protamine (300 IU/kg) was administered. To maintain systolic blood pressure above 70 mm Hg and hemoglobin greater than 6 mg/dL, both volume substitution and continuous noradrenaline infusion were administered. Samples from the left anterior wall were harvested for determination of high-energy phosphates, miRNAs, histology, and electron microscopy. The pigs were humanely killed with high-dose pentobarbital.

Cardioplegic Solutions

The basic composition of STH-Pol-B was 1.0 mmol/L esmolol (Baxter, Vienna, Austria), 0.5 mmol/L adenosine (Sigma-Aldrich, St Louis, Mo), and 10.0 mmol/L magnesium gluconate (G.L. Pharma GmbH, Lannach, Austria) in a total volume of 1000 mL of Ringer's solution. Pig blood (500 mL) was mixed with this crystalloid solution (1:2; total volume: 1500 mL) immediately before administration. STH2 (NaCl: 110.0 mmol/L, NaHCO₃: 10.0 mmol/L, KCl: 16.0 mmol/L, MgCl₂: 16.0 mmol/L; CaCl₂: 1.2 mmol/L) was provided by the hospital pharmacy of the General Hospital Linz, Austria, and was mixed (1:2; total volume: 1500 mL) with 500 mL of pig blood. After aortic crossclamping, 1000 mL of the respective blood cardioplegia was infused with a pressure of 60 mm Hg and a temperature of 4°C; after 30 minutes of ischemia, an additional 500 mL of the

FIGURE 1. Experimental protocol. After baseline hemodynamic and echocardiography measurements, aortic crossclamping was performed followed by 60 minutes of ischemia, 60 minutes of on-pump and, finally, 90 minutes of off-pump reperfusion. Before the pigs were humanely killed, echocardiography was repeated. A, Administration of the first dose of STH-Pol-B or STH2-B (1000 mL after aortic crossclamping). B, Administration of the second dose of STH-Pol-B or STH2-B during ischemia (500 mL). Pig blood was mixed with the crystalloid solution in a ratio of 1:2—see the Methods. The indicated times refer to sampling points. *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2.

cardioplegia solutions was infused. The final molar concentrations of both cardioplegia solutions are presented in [Table E1](#).

Hemodynamic Evaluation

Heart rate (HR), arterial pressure (AP), right atrial pressure, left ventricular pressure (LVP), and cardiac output (CO) were continuously measured. Coronary flow in the left anterior descending coronary artery and wedge pressure were recorded at baseline (before start of CPB) and at 1, 5, 15, 30, 60, 90, 120, and 150 minutes of reperfusion. Echocardiographic evaluation—ejection fraction, fractional shortening—was performed at baseline and before sacrifice. External heart work (EHW) was calculated by $(CO \times \text{systolic LVP})$ for each time point.

Biochemical Analyses

CK-MB, Troponin I, Lactate, Oxygen Consumption, Malondialdehyde Assessment, and High-Energy Phosphates.

Energy status are described in the [Appendix E1](#).

Histology

Histology and electron microscopy are described in the [Appendix E1](#).

MiRNA and Quantitative Polymerase Chain Reaction Analyses

MiRNA and quantitative polymerase chain reaction analyses are described in detail in the [Appendix E1](#).

Statistical Analysis

Graphs were generated with GraphPad Prism (7.04; GraphPad Software, La Jolla, Calif), and statistical analysis was performed using IBM SPSS Statistics 24 (IBM Corp, Armonk, NY). Sample size calculation was based on the primary outcome variable and was performed with the sample size calculator available at <http://clincalc.com/Stats/SampleSize.aspx>. Based on a previous study in rats by Fujii and Chambers,⁸ we expected a standard deviation of 3 units. We defined a minimally relevant group difference as 5 units. With a power of 80%, accepting the probability of a type I error of 5%, 6 animals per group were needed. As a safety margin, we aimed for 7 pigs per group. One animal had to be excluded due to a cannulation problem.

To estimate the difference between animals receiving STH-Pol-B or STH2-B regarding the dependent variables, mixed linear models were

applied. First, data were inspected visually for approximate normal distribution in both groups at all time points, and right-skewed data were log10-transformed. Data are given as mean and standard deviation in case normal distribution could be assumed, right-skewed data as given as geometric mean with its 95% confidence interval. The factor “group” with the 2 levels STH-Pol-B and STH2-B was specified as a fixed between-subjects factor, the factor “time” with the respective time points as levels was specified as fixed within-subjects factor. Further, each pig was included as a level of a random factor. To adjust the models for pretreatment differences, the baseline values were included as a covariate. The models were estimated using the restricted maximum likelihood method. After we chose adequate covariance-structures based on the smallest Akaike information criterion value, a time by group interaction was tested. In case of a significant interaction, contrasts were used to estimate group differences at each time point. Otherwise, the interaction term was dropped from the model, and the main effect of “group” was interpreted as group difference that applies to all time points. All reported *P* values are result of 2-sided tests. *P* values of .05 or less were considered significant.

Pearson correlation coefficient was used to analyze the correlation between troponin I and miR-122. Binary events were compared with Fisher exact test. Metric values are presented as absolute values or as mean $\pm$ standard deviation, ordinal variables are shown as median with interquartile range. A *P* value of $<.05$ was considered significant. For miRNA analysis, differentially expressed miRNAs were identified based on the classic statistical functions available under publicly available R-package EdgeR.⁹ We used the nontransformed miRNA read counts for analysis under EdgeR. Initially, to account for technical factors influencing miRNA detection, EdgeR was used for adjusting to effects resulting from varying sequencing depths through normalization to the total library size (automatic function). Next, to account for varying RNA composition, a model-based scale normalization was performed for every sample by calculating the trimmed means of M-values. For differential expression analysis, the glm functions available under EdgeR were used together with likelihood ratio tests. The obtained *P* values were corrected for multiple testing by applying the Benjamini–Hochberg method on *P* values to control false-discovery rate. Arterial CK-MB release was defined as primary endpoint; therefore, we took mean values from Fujii and Chambers⁸ and aimed for a comparable reduction in the STH-Pol-B versus STH2-B group. All secondary outcome measures were not adjusted for multiplicity because of the exploratory nature of this study and have to be interpreted accordingly. Electron microscopy data were analyzed using a Mann–Whitney *U* test with an exact *P* value due to limited sample size. The

damage categories no, slight, moderate, severe, and irreversible damage were coded as 0, 1, 2, 3, and 4, respectively, allowing for coding of intermediate evaluations as “slight to moderate” corresponding to 2.5.

RESULTS

Animal Characteristics

Baseline values are depicted in Table 1. Thirteen pigs were included in the study (STH-Pol-B: $n = 7$, 52 ± 4 kg, STH2-B: $n = 6$, 62 ± 4 kg; $P = .1$). Time to asystole was similar in both groups (STH-Pol-B: 154 ± 65 seconds vs STH2-B: 170 ± 36 seconds; $P = .84$). All hearts were arrested during the first application of cardioplegia. Five STH-Pol-B and three STH2-B pigs had ventricular fibrillation during early reperfusion. Numbers of applied defibrillations were comparable in both groups ($P = .071$; Figure E1). None of the pigs required temporary pacemaker stimulation during reperfusion.

Hemodynamic Data

Baseline hemodynamics are depicted in Table E2. There were no differences between groups for any parameter at baseline. Ejection fraction (STH-Pol-B vs STH2-B: 58 ± 4 vs $63 \pm 2\%$; $P = .46$) and fractional shortening (STH-Pol-B vs STH2-B: 31 ± 2 vs $33 \pm 2\%$; $P = .50$) were comparable at baseline and at the end of the experiment. During reperfusion, HR ($P = .18$), systolic AP ($P = .32$), right atrial pressure ($P = .296$), CO ($P = .282$), left ventricular end-diastolic pressure ($P = .411$), and noradrenaline support ($P = .702$; Table 2 and Figure E2) were comparable between both groups, whereas the following parameters were significantly increased in STH-Pol-B versus STH2-B: diastolic AP ($P = .032$), mean arterial pressure ($P = .007$; Figure 2, A), systolic LVP ($P < .001$; Figure 2, B), stroke volume ($P = .015$; Figure 2, C), external heart work (EHW; $P = .012$; Figure 2, D), left ventricular end systolic pressure ($P = .01$, Figure 3, A), and coronary flow ($P = .03$; Figure 3, B). Wedge pressure was markedly lower in STH-Pol-B after weaning ($P = .014$; Figure 3, C). Left ventricular

contractility described by dp/dt_{max} was significantly greater in STH-Pol-B ($P = .027$; Figure 3, D), whereas dp/dt_{min} was comparable in both groups ($P = .608$).

Biochemical Data

During reperfusion, arterial CK-MB ($P = .283$), troponin I ($P = .543$), and malondialdehyde ($P = .709$; Figure E3) were similar (Table 2), whereas coronary CK-MB showed a tendency to be significantly lower in STH-Pol-B-treated hearts ($P = .058$; Table 2). On-pump arterial and coronary lactate levels were slightly, but not significantly, greater (arterial lactate: $P = .501$; coronary lactate: $P_{interaction} = .005$; Table 2) in the STH2-B group; coronary pH was correspondingly and significantly acidotic in STH2-B at 30 minutes ($P = .03$) and 60 minutes ($P = .01$) of reperfusion (Table 2). In addition, there was a trend ($P = .06$; Table 3) toward increased myocardial oxygen content, in STH-Pol-B, whereas myocardial oxygen consumption and myocardial oxygen extraction were similar in both groups (both n.s., data not shown).

Arterial CK-MB ($P = .283$) as well as troponin I ($P = .534$) were comparable in both groups (Table 3). High-energy phosphates were analyzed at the end of the experiment. There was no significant difference in levels of hypoxanthine between groups (Table E3).

Electron Microscopy Data

None of the hearts in either group showed severe to irreversible damage. In STH-Pol-B, 2 samples showed no damage, 1 slight damage, 1 slight-to-moderate damage, and 3 moderate damage. In STH2-B, 1 sample showed no damage, 1 no to slight damage, 2 slight damage, and 3 samples showed slight-to-moderate damage indicating similar protection with both cardioplegic solutions ($P = .42$; Figures E4 and E5).

Differential Expression of miRNAs in Left Ventricle (LV) Tissue Samples

For next-generation sequencing, myocardial LV samples were taken at the end of the experiment to identify the miRNAs expression pattern. Overall, 238 miRNAs were detected in all samples with a minimum abundance of 1 tags per million (TPM). Expression of top-regulated miRNAs ($P < .3$) are shown in Table E4. Principal component analysis suggested that miRNA expression in the LV was not primarily influenced by the type of cardioplegic solution. Interestingly, elevated expression of ssc-miR-122 correlated positively with troponin-I after 150 minutes of reperfusion, irrespectively of the type of cardioplegic solution applied. The TPM values of 2 miRNAs, ssc-miR-708-5p (STH-Pol-B vs STH2-B: 26 ± 13 vs 42 ± 21 TPM; $P = .019$) and ssc-miR-122 (STH-Pol-B vs STH2-B: 25 ± 35 vs 10 ± 8 TPM; $P = .046$), were significantly different between STH-Pol-B and STH2-B (Figure 4, A).

TABLE 1. Animal characteristics and events

Group	STH-Pol-B (n = 7)	STH2-B (n = 6)	P value
BW, kg	52 ± 9	62 ± 11	.1
HW, g	$225 \pm 28^*$	$284 \pm 31^*$	.01
Heart wet/dry ratio, %	20.0 ± 0.7	19.3 ± 0.8	.11
Time to asystole, s	154 ± 173	170 ± 88	.84
VF	5	3	1.00
PM (%)	0 (0)	0 (0)	1.0

Values are given as mean $\pm$ standard deviation. STH-Pol-B, New St Thomas' Hospital Polarizing blood cardioplegia; STH2-B, St Thomas' Hospital blood cardioplegia No. 2; BW, body weight; HW, heart weight; VF, ventricular fibrillation; PM, number of animals that demanded a pacemaker during reperfusion. * $P < .05$.

TABLE 2. Parameters during on-pump reperfusion phase

Index	Group	Time points						$P_{\text{interaction}}$	P_{group}	P_{time}
		Baseline	1 min	5 min	15 min	30 min	60 min			
HR, bpm	STH-Pol-B	113 ± 22	82 ± 22	94 ± 20	100 ± 25	108 ± 19	117 ± 20	.567	.18	.027
	STH2-B	101 ± 8	82 ± 19	96 ± 6	93 ± 15	90 ± 16	98 ± 18			
CF, mL/g/min	STH-Pol-B	137 ± 110	345 ± 288	320 ± 215	302 ± 242	310 ± 220	249 ± 178	.687	.03	.084
	STH2-B	131 ± 68	196 ± 60	155 ± 49	195 ± 99	174 ± 88	191 ± 130			
Noradrenaline, $\mu\text{g/kg/min}$	STH-Pol-B	0.16 ± 0.39	0.93 ± 0.27	1.05 ± 0.54	1.05 ± 0.54	1.05 ± 0.54	1.56 ± 0.70	.496	.702	.018
	STH2-B	0.09 ± 0.23	0.99 ± 0.80	1.02 ± 0.83	1.00 ± 1.82	1.23 ± 0.87	1.29 ± 0.58			
Arterial CK-MB, U/L	STH-Pol-B	184 ± 75	255 ± 62	254 ± 64	261 ± 84	286 ± 68	338 ± 73	.310	.283	.000
	STH2-B	246 ± 75	338 ± 95	343 ± 95	373 ± 105	384 ± 80	476 ± 108			
Coronary CK-MB, U/L	STH-Pol-B	205 ± 69	225 ± 66	243 ± 44	270 ± 45	273 ± 65	334 ± 87	.810	.058	.000
	STH2-B	266 ± 83	341 ± 97	349 ± 98	375 ± 103	394 ± 87	467 ± 90			
Arterial troponin I*	STH-Pol-B	1.79 (0.75-4.26)	2.28 (1.26-4.1)	2.35 (1.28-4.35)	2.73 (1.54-4.83)	3.84 (1.93-7.64)	9.33 (4.55-19.14)	.93	.534	.000
	STH2-B	2.57 (1.31-5.05)	2.81 (1.18-6.71)	3.17 (1.36-7.36)	3.22 (1.44-7.20)	4.04 (1.67-9.77)	10.41 (4.70-23.06)			
Malondialdehyde, nmol/mL	STH-Pol-B	6.03 ± 1.78	8.81 ± 2.82	9.28 ± 2.38	9.08 ± 3.69	n/a	9.76 ± 4.48	.389	.709	.888
	STH2-B	7.50 ± 0.87	9.32 ± 3.22	8.53 ± 2.61	8.58 ± 0.98	n/a	11.36 ± 3.06			
Arterial lactate, mmol/dL	STH-Pol-B	4.4 ± 0.8	4.0 ± 0.8	4.2 ± 1.2	4.0 ± 1.4	4.0 ± 1.4	3.9 ± 1.5	.074	.501	.356
	STH2-B	5.3 ± 2.3	4.5 ± 1.3	4.6 ± 1.4	4.8 ± 1.5	5.0 ± 1.7	5.6 ± 2.0			
Coronary lactate, mmol/dL	STH-Pol-B	3.2 ± 1.4	4.9 ± 1.5	4.2 ± 1.1	4.1 ± 1.4	4.3 ± 1.5	4.1 ± 1.1	.005		
	STH2-B	3.6 ± 1.0	4.5 ± 1.2	4.6 ± 1.4	4.7 ± 1.4	5.1 ± 1.7	5.7 ± 2.0			
Coronary pH	STH-Pol-B	7.34 ± 0.06	7.34 ± 0.10	7.35 ± 0.10	7.36 ± 0.09	7.37 ± 0.08*	7.41 ± 0.06*	.003		
	STH2-B	7.35 ± 0.03	7.27 ± 0.08	7.27 ± 0.10	7.25 ± 0.10	7.24 ± 0.10*	7.28 ± 0.09*			
Coronary venous oxygen content, mL O ₂ /mL	STH-Pol-B	247 ± 99	490 ± 165	465 ± 133	414 ± 94	426 ± 86	483 ± 134	.328	.069	.050
	STH2-B	209 ± 98	338 ± 167	328 ± 181	317 ± 144	290 ± 135	434 ± 63			

Values are mean ± standard deviation unless noted. Groups were compared in the following biochemical parameters: arterial and coronary CK-MB, malondialdehyde, arterial and coronary lactate, as well as coronary pH. P values are given for interaction ($P_{\text{interaction}}$), between groups (P_{group}), and within groups (P_{time}). HR, Heart rate; STH-Pol-B, new St Thomas' Hospital Polarizing blood cardioplegia; STH2-B, St Thomas' Hospital blood cardioplegia No. 2; CF, coronary flow; CK-MB, creatine kinase-muscle/brain. *Original data were right-skewed data; therefore, geometric means with 95% confidence interval are given.

Interestingly, there was a significant positive correlation between ssc-miR-122 expression and troponin I after (150 minutes of reperfusion), irrespectively of the type of cardioplegic solution applied ($P < .0001$, $r = 0.9061$; Figure 4, B). In addition, we performed network analysis (Mirnet) to assess the intersection between experimentally verified targets of miR-122-5p and miR-708-5p. We observed only a very small overlap in experimentally verified genes (5 genes: SRF, SLC7A5, PRSS16, YKT6, and FOXJ3; Figure 4, C) between both miRNAs. In addition, subsequent analysis for the assessment of proinflammatory

cytokines (tumor necrosis factor [TNF]-alpha, interleukin [IL]-6, and high mobility group box 1 [HMGB1]) and apoptosis (Caspase-3) related genes showed no differences between the groups (TNF-alpha: $P = .78$; IL-6: $P = .85$; HMGB1: $P = .84$; Caspase-3: $P = .09$; Figure E6).

DISCUSSION

This study characterized the effects of low-dose polarized STH-Pol-B during prolonged on-pump and off-pump reperfusion after 60 minutes of cardiac arrest. The data reveal similar myocardial protection with STH-Pol-B

FIGURE 2. Hemodynamic data were recorded at baseline and every 30 minutes during off-pump reperfusion. Mean arterial pressure (A), systolic left ventricular pressure (LVP; B), stroke volume (C), and external heart work (D) were significantly increased in STH-Pol-B. Values are mean $\pm$ standard deviation; P values are given for interaction (P_i) and between groups (P_g). *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2.

compared with STH2-B. During 60 minutes of on-pump reperfusion, arterial and coronary CK-MB were comparable. Notably, hemodynamics were significantly improved after weaning from CPB: diastolic AP, mean arterial pressure, systolic LVP, left ventricular end-systolic pressure, CO, stroke volume, EHW, and dp/dt_{max} (indicating better systolic function) were significantly greater in STH-Pol-B. Wedge pressure was decreased after weaning, indicating improved diastolic function. In addition, the present study for the first time describes the pattern of miRNAs in pig's myocardium following CPB. The data supports noninferiority of polarized arrest with STH-Pol-B compared with conventional depolarized arrest with STH2-B in a porcine model.

Beta blockers in general¹⁰ and esmolol in particular have already been used clinically as continuous coronary perfusion during cardiac surgery.¹¹ Esmolol has a short half-life (approximately 9 minutes) and is hydrolyzed by red blood cell esterases,¹² which rapidly reverse negative inotropic effects during reperfusion. We showed

comparable echocardiographic measurements at baseline and at the end of the experiments, next to hemodynamic improvements in STH-Pol-B. Esmolol did clearly not dampen hemodynamics since parameters such as HR, systolic AP, and LVP were significantly improved in STH-Pol-B. Stable hemodynamic conditions during on-pump reperfusion and after weaning are important requirements in fragile hearts during cardiac surgery.

Adenosine prevents Ca^{2+} overload and is cardioprotective during hyperkalemia-induced cardioplegic arrest by lessening the risk for ventricular dysfunction.¹³ Besides the beneficial hemodynamic effects, STH-Pol-B maintained normal pH values in the coronary blood whereas in STH2-B hearts, acidic pH values were observed during reperfusion. By inhibition of mitochondrial permeability transition formation¹⁴ moderate acidosis during ischemia is cardioprotective; however, the following normalization of pH during reperfusion induces the opening of mitochondrial permeability transition and leads to apoptosis.¹⁴ Changes in pH should be avoided during ischemia-reperfusion.

FIGURE 3. Hemodynamic data were recorded at baseline and every 30 minutes during off-pump reperfusion. Left ventricular end systolic pressure (LVESP; A) as well as coronary flow (B) were significantly elevated, wedge pressure (C) was significantly alleviated and dp/dt_{max} (D) was significantly improved in STH-Pol-B. Values are mean $\pm$ SD; P values are given for interaction (P_i) and between groups (P_g). STH-Pol-B, New St Thomas' Hospital Polarizing blood cardioplegia; STH2-B, St Thomas' Hospital blood cardioplegia No. 2.

Therefore, our preserved coronary blood pH values underline the biochemical advantages of polarized arrest.

Recently, Aass and colleagues⁷ performed a comparable study about the effect of polarized cardioplegia on energy metabolism and cardiac function. They showed an improved cardiac index after 150 minutes of reperfusion. The most important findings were increased levels of phosphocreatine and ATP during late ischemia and early phase of reperfusion in hearts preserved with STH-Pol-B. After 180 minutes of reperfusion, these differences were no longer detectable. The major difference as compared with the present study was the temperature of cardioplegia during application (4°C vs 12°C) and the time of on-pump reperfusion (60 minutes vs 10 minutes). This longer reperfusion period of 60 minutes was chosen since it is known that controlled reperfusion is beneficial in hearts with reduced function,¹⁵ which is—next to old age—a predictor for increased inotropic support after weaning from CPB.¹⁶ The main reason for using a lower temperature was based on previous studies that demonstrated the

efficacy and cardioprotective effect of administration at low temperature.¹⁷ These differences might explain the enhanced cardiac function and reduction in myocardial damage in comparison with the work of Aass and coworkers.⁷

In addition, we investigated expression levels of miRNAs in LV tissue samples. Previously, only a few studies have investigated the expression pattern of miRNAs in the setting of CPB. However, these previous studies investigated the expression of miRNAs in CPB using microarray analysis with a preselected number of miRNAs. To the best of our knowledge, no study so far identified miRNAs in myocardial LV tissue samples obtained from hearts subjected to cardioplegia and reperfusion. With next-generation sequencing, overall 238 miRNAs were identified to be robustly expressed in LV tissue; only 2 miRNAs (miR-708-5p and miR-122) showed statistically different expression levels between the 2 groups. A recent pioneering study identified miR-708 as an anti-inflammatory miRNA in endothelial and vascular smooth muscle cells, and there is

TABLE 3. Hemodynamics after weaning from CPB

Index	Group	Time points				$P_{\text{interaction}}$	P_{group}	P_{time}
		Baseline	90 min	120 min	150 min			
HR, bpm	STH-Pol-B	113 ± 22	117 ± 24	109 ± 9	113 ± 10	.567	.18	.027
	STH2-B	101 ± 8	99 ± 6	108 ± 28	104 ± 16			
Systolic AP, mm Hg	STH-Pol-B	72 ± 16	115 ± 24	113 ± 24	113 ± 33	.541	.127	.424
	STH2-B	77 ± 13	93 ± 26	89 ± 32	68 ± 37			
Diastolic AP, mm Hg	STH-Pol-B	42 ± 11	47 ± 9	37 ± 10	37 ± 8	.092	.032	.03
	STH2-B	46 ± 10	39 ± 9	39 ± 18	24 ± 9			
MAP, mm Hg	STH-Pol-B	55 ± 15	68 ± 16	54 ± 12	59 ± 17	.09	.007	.003
	STH2-B	60 ± 9	56 ± 17	52 ± 27	32 ± 12			
RAP, mean, mm Hg	STH-Pol-B	6 ± 5	8 ± 5	7 ± 4	7 ± 4	.149	.296	.291
	STH2-B	8 ± 2	9 ± 2	8 ± 3	10 ± 6			
Systolic LVP, mm Hg	STH-Pol-B	77 ± 16	113 ± 23	96 ± 9	97 ± 13	.607	.000	.083
	STH2-B	83 ± 6	98 ± 27	79 ± 11	73 ± 11			
SV, mL/g/beat	STH-Pol-B	189 ± 54	274 ± 79	201 ± 60	180 ± 54	.870	.015	.001
	STH2-B	165 ± 31	245 ± 60	129 ± 39	136 ± 30			
CO, mL/g hw	STH-Pol-B	20 ± 3	20 ± 3	18 ± 4	17 ± 5	.347	.282	.005
	STH2-B	17 ± 3	17 ± 4	13 ± 3	15 ± 2			
EHW, mL/g hw	STH-Pol-B	1.60 ± 0.46	2.28 ± 0.43	1.76 ± 0.45	1.75 ± 0.72	.889	.012	.044
	STH2-B	1.38 ± 0.28	1.56 ± 0.53	0.97 ± 0.34	0.98 ± 0.28			
LVESP, mm Hg	STH-Pol-B	82 ± 12	114 ± 25	97 ± 8	99 ± 18	.25	.01	.066
	STH2-B	79 ± 13	106 ± 36	84 ± 18	65 ± 23			
LVEDP, mm Hg*	STH-Pol-B	12.38 (10.09-15.19)	17.72 (11.88-26.42)	14.83 (11.65-18.86)	13.12 (10.54-16.33)	.073	.411	.474
	STH2-B	9.44 (2.97-29.96)	14.67 (13.13-16.4)	14.74 (13.06-16.63)	17.29 (11.67-25.61)			
CF, mL/g/min	STH-Pol-B	137 ± 110	264 ± 167	241 ± 147	242 ± 127	.687	.03	.084
	STH2-B	131 ± 68	181 ± 68	128 ± 36	132 ± 44			
Wedge, mm Hg	STH-Pol-B	10 ± 3	11 ± 2	10 ± 3	10 ± 3	.205	.014	.573
	STH2-B	11 ± 3	14 ± 4	14 ± 4	14 ± 3			
dp/dt _{max} , mm Hg/s	STH-Pol-B	1387 ± 474	3632 ± 508	2914 ± 834	3206 ± 1226	.44	.027	.114
	STH2-B	1038 ± 583	2726 ± 918	2437 ± 1032	1957 ± 495			
Arterial CK-MB, U/L	STH-Pol-B	184 ± 75	472 ± 116	482 ± 141	494 ± 151	.310	.283	.000
	STH2-B	246 ± 75	526 ± 91	516 ± 111	494 ± 111			
Arterial troponin-I*	STH-Pol-B	1.79 (0.75-4.26)	31.64 (15.66-63.95)	44.73 (20-100.1)	50.21 (16.38-153.9)	.93	.534	.000
	STH2-B	2.57 (1.31-5.05)	22.65 (11.22-45.74)	34.18 (20.58-56.79)	45.3 (34.14-60.1)			

(Continued)

TABLE 3. Continued

Index	Group	Time points				<i>P</i> _{interaction}	<i>P</i> _{group}	<i>P</i> _{time}
		Baseline	90 min	120 min	150 min			
Arterial lactate, mmol/dL	STH-Pol-B	4.4 ± 0.8	3.9 ± 1.5	4.4 ± 2.5	4.8 ± 3.5	.074	.501	.356
	STH2-B	5.3 ± 2.3	6.1 ± 1.6	7.1 ± 2.6	9.1 ± 2.9			
Noradrenaline, µg/kg/min	STH-Pol-B	0.16 ± 0.39	1.70 ± 1.08	1.66 ± 1.01	1.99 ± 1.11	.496	.792	.018
	STH2-B	0.09 ± 0.23	1.29 ± 0.58	1.81 ± 0.62	1.94 ± 1.28			

After weaning from CPB, the following parameters were recorded: HR, CF, systolic and diastolic AP, MAP, RAP, LVP, SV, CO, EHW, LVESP and LVEDP, wedge pressure, dp/dt_{max}, CF, and noradrenaline administration. Furthermore, groups were compared in the following biochemical parameters: arterial CK-MB and arterial lactate. Values are mean ± standard deviation unless noted. *P* values are given for interaction (*P*_{interaction}), between groups (*P*_{group}), and within groups (*P*_{time}). HR, Heart rate; *STH-Pol-B*, new St Thomas’ Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas’ Hospital blood cardioplegia No. 2; AP, systolic and diastolic arterial pressure; MAP, mean arterial pressure; RAP, right atrial pressure; LVP, systolic left ventricular pressure; SV, stroke volume; CO, cardiac output; EHW, external heart work; LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure wedge pressure; CF, coronary flow; CK-MB, creatine kinase-muscle/brain. *Original data were right-skewed data; therefore, geometric means with 95% confidence interval are given.

substantial evidence that inflammation contributes to impaired cardiac and vascular function during CPB.¹⁸ Therefore, we performed subsequent analysis for the assessment of proinflammatory cytokines (TNF-alpha, IL-6, and HMGB1) and apoptosis (Caspase-3) related genes. There

were no differences between the groups (Figure E6). In addition, upregulation of miR-708 expression following ischemia–reperfusion may have deleterious effects on cardiac contractility and intracellular Ca²⁺ homeostasis by acting on cardiac neuronal nitric oxide synthase.¹⁹ In line

FIGURE 4. Distinct myocardial microRNA expression profiles of STH2-B group compared with the STH-Pol-B group. A, Volcano plot of the miRNA expression levels in STH2-B and STH-Pol-B group in the discover set. The horizontal line represents a *P* value of .05 and miR-122-5p and miR-708-5p plots (red) represent the differentially expressed miRNAs with statistical significance. B, miR-122 expression in the left ventricle samples correlates with peripheral troponin I levels, independent of the cardioplegic solution (each plot represents an individual pig). C, miRNA (mir-122 and mir-708)-mRNA network analysis and intersection between experimentally verified target genes (SRF, SLC7A5, PRSS16, YKT6, FOXJ3) of miR-122-5p and miR-708-5p overlapping. *STH-Pol-B*, New St Thomas’ Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas’ Hospital blood cardioplegia No. 2.

with this, downregulation of mir-708 expression in STH-Pol-B group might contribute to improve LV systolic function. MiR-122 has been shown that its levels positively correlate to cardiac troponin-I following acute myocardial infarction and progression of heart failure.²⁰ Accordingly, we found that miR-122 expression in the LV samples correlates with peripheral troponin-I levels, independent of the cardioplegic solution. This result indicates that miR-122 might be a potential biomarker of ischemia–reperfusion injury following CPB.

Previous clinical studies showed clinical efficacy of polarized arrest (microplegia: lidocaine, magnesium, and adenosine) with improved hemodynamics and reduced reperfusion damage in patients with unstable angina.²¹ It is of interest that the principal component of the described “polarizing” solution is lidocaine, a Na-channel blocker

with a considerable half-life of 90 to 120 minutes with normally functioning liver and kidney. In contrast, the half-life of esmolol is about 9 minutes. We think that this makes esmolol (which acts to inhibit both the Ca-channel and the Na-channel) a safer drug to use in the acute setting of cardiac surgery, as it is extremely unlikely that there will be any residual drug remaining in the systemic circulation at the end of CPB, in contrast to the highly likely scenario that lidocaine at relatively high concentrations may remain systemically. Furthermore, STH-Pol-B is not comparable with reduced volume solutions; in fact, this form of application might not be sufficient for every patient.²²

The present study has the following limitations. Experiments were performed on healthy pigs with an ischemia time of 60 minutes to provide comparable experimental conditions with our collaborators in Norway.⁷ Animals

FIGURE 5. The new St Thomas' Polarized Hospital blood cardioplegia consists of 3 main components (esmolol, magnesium, and adenosine) and was compared with depolarized St Thomas' No. 2 blood cardioplegia in a cardiopulmonary bypass model in pigs. Results showed improved hemodynamic recovery and similar reperfusion injury, which supports noninferiority of polarized arrest.

VIDEO 1. Bruno K. Podesser, senior author, and David Santer, first author, provide a brief introduction on the hypothesis, design, and results of the study. Video available at: [https://www.jtcvs.org/article/S0022-5223\(18\)33254-9/fulltext](https://www.jtcvs.org/article/S0022-5223(18)33254-9/fulltext).

underwent on-pump reperfusion of 60 minutes to guarantee controlled reperfusion under comparable flow rates, pressures, and hemoglobin. Under these standardized conditions, analysis of cardiac enzymes from coronary sinus blood was performed. Besides CK-MB, we also analyzed the more sensitive parameter troponin I, which also showed comparable results in both groups. It is important to mention that we were aware that esmolol is hydrolyzed by red blood cell esterases. Therefore, the crystalloid components of the cardioplegia were placed on ice and mixed with the ice-cold blood of the pig just before administration. Although a sample size was defined, it is possible that between-group differences might be caused by a sampling error due to the low number of pigs. In addition, this study does not provide pressure–volume relationships, since measurements were performed with a pressure-tip catheter. Since correction for multiple testing has not been performed, the miRNA findings in this study are of exploratory nature and require further replication to verify their relevance for the regulation of gene activity in hearts undergoing cardioplegic arrest.

In summary, the functional, biochemical and molecular data of this study suggests the noninferiority as well as potential benefits of a novel hypothermic blood-based polarized St Thomas' Hospital cardioplegic solution in a clinically relevant model of CPB in pigs (Figure 5 and Video 1).

Conflict of Interest Statement

Matthias Hackl is co-founder of TAMiRNA. David J. Chambers is co-inventor of the STH-Pol solution. All other authors have nothing to disclose with regard to commercial support.

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Key Words: cardioplegia, St Thomas' Hospital No. 2, polarizing cardioplegia, animal study, micro RNA, ischemia-reperfusion, cardiopulmonary bypass, blood cardioplegia, cardioprotection

APPENDIX E1. SUPPLEMENTAL METHODS

Protocol

For premedication, ketamine (15 mg/kg intramuscularly), acepromazine (1.3 mg/kg), and atropine (0.5 mg) were used. Animals were anesthetized (2.5 mg/kg propofol, 15 mg piritramide, and 20 mg rocuronium bromide), intubated, and ventilated. Anesthesia was maintained with piritramide (1.875 mg/kg/h), rocuronium bromide (3.7 mg/kg/h), and propofol (10 mg/kg/h). Pressure monitoring was performed via the left femoral artery, and a central venous catheter was placed into the left external jugular vein for venous access. After sternotomy, pigs were heparinized (300 IU/kg), the ascending aorta (Optisite arterial cannula; Edwards Lifesciences Corp, Irvine, Calif) and the right atrium (Trim Flex venous cannula; Edwards Lifesciences Corp) were cannulated, and the pigs were put on normothermic cardiopulmonary bypass (Stockert SIII Heart Lung System; PERFUSION.COM, Inc, Fort Myers, FL). Additional monitoring included a Swan–Ganz oximetry thermodilution catheter (741HF75P; Edwards Lifesciences Corp) for pulmonary capillary wedge pressure and cardiac output, ultrasound flow probe for coronary flow of the left anterior descending coronary artery (LAD), a pressure tip catheter for left ventricular pressure (Millar Instruments, Houston, Tex), and a coronary sinus catheter to sample coronary effluent during first 60 minutes of ischemia and reperfusion.

High-Energy Phosphates: Energy Status

Samples from the mid-anterior wall of the left ventricle next to the LAD were snap frozen with precooled tongs (liquid nitrogen) and the biopsies stored in liquid nitrogen before high-energy phosphates analysis. The sample preparation and high-performance liquid chromatography (HPLC) measurement of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate, and phosphocreatine, as well as hypoxanthine and xanthine were performed as previously described.^{E1,E2} A piece of frozen tissue (50–100 mg) was homogenized with a micro-dismembrator (Braun, Melsungen, Germany) and deproteinized with 400 μ L of 0.4 mol/L perchloric acid. After centrifugation (12,000g) 200 μ L of the acid extract was neutralized with 20 to 25 μ L of 2 mol/L potassium carbonate (4°C). The supernatant (20 μ L injection volume) obtained after centrifugation was used for HPLC analysis.

To summarize in brief, separation was performed on a Hypersil ODS column (5 μ m, 250 mm $\times$ 4 mm inner diameter) using a L-2200 autosampler, 2 L-2130 HTA pumps, and an L-2450 diode array detector (VWR International, Wien, Austria, and Hitachi HTA, Schaumburg, Ill). Detector signals (absorbance at 214 nm and 254 nm) were recorded and the program EZchrom Elite (VWR International) was used for data acquisition and analysis.

Energy charge was calculated as $(\text{ATP} + \frac{1}{2} \text{ADP}) / (\text{ATP} + \text{ADP} + \text{adenosine monophosphate})$. The pellets of the acid extract were dissolved in 1 mL of 0.1 mol/L sodium hydroxide and diluted 1:10 with physiologic saline for protein determination (BCA Protein Assay; Thermo Fisher Scientific, Inc, Rockford, Ill).

Histology

Myocardial samples (1 $\times$ 1 $\times$ 1 mm) were cut with a surgical blade in a no-touch technique from a specimen collected from the mid-anterior wall of the left ventricle next to the LAD and preserved in buffer (4% paraformaldehyde in 0.1 M sodium cacodylate buffer) for electron microscopy. Electron microscopy was performed as described elsewhere.^{E3} Ultrastructural integrity was evaluated in samples from the left anterior wall with transmission electron microscopy using 3 randomly selected fields in a blinded manner per animal. Ischemic damage was graded according to the following scheme based on mitochondrial damage^{E3}: (0) no visible damage: normal matrix granules; (1) slight damage: loss of matrix granules, light clearing of matrix; (2) moderate damage: moderate clearing of matrix, moderate swelling and partial fragmentation of cristae; (3) severe damage: severe matrix clearing, severe swelling and loss of cristae; and (4) irreversible damage: amorphous dense granules. Representative micrographs of Grade 0 and Grade 2 are shown in Figure E4.

MicroRNA (miRNA) Analysis

Samples from the left anterior wall were stored at -80°C before miRNAs analyses. Total RNA was extracted from homogenized heart tissue using the miRNeasy purification kit (QIAGEN, Hilden, Germany) and was quality checked for RNA integrity using the RNA 6000 bioanalyzer assay (Agilent, Carpinteria, Calif) and spectrophotometric RNA quantification (NanoDrop; Thermo Fisher Scientific, Inc). Equal amounts of total RNA (250 ng) were used for small RNA library preparation using the Clean-Tag ligation kit (TriLink, San Diego, Calif). Adapter-ligated libraries were amplified using barcoded Illumina reverse primers in combination with the Illumina forward primer. Libraries were pooled at equimolar rates on the basis of a DNA-1000 bioanalyzer run (Agilent) and sent for sequencing to Exiqon (Vedbæk, Denmark). The small RNA library pool was sequenced on an Illumina NextSeq 500 with 50-bp cycle length. Reads were adapter trimmed and filtered for low-quality reads ($Q < 30$). MiRNA annotation was performed on the basis of sequence alignments against the genome reference and miRBase release 21.^{E4} Read counts were normalized to the total number of reads detected per sample to obtain the “tags per million” for each miRNA and sample. Exploratory analysis was performed using ClustVis,^{E5} and differential expression analysis was performed using

the EdgeR^{E6} package under R/Bioconductor. In addition, Mirnet (www.mirnet.ca) was used to analyze the intersection between experimentally verified targets of miR-122-5p and miR-708-5p. Based on the absence of specific data for *Sus scrofa domestica* (pig), human data were used as reference. Subsequently, analysis for the assessment of proinflammatory cytokines (tumor necrosis factor- α , interleukin-6, and high mobility group box 1) and apoptosis (caspase-3) related genes was performed.

Creatine Kinase-MB, Troponin-I, Lactate, Oxygen Consumption, and Malondialdehyde Assessment

Arterial blood samples were drawn at baseline, 1, 5, 15, 30, 60, 90, 120, and 150 minutes of reperfusion. During cardiopulmonary bypass, venous samples were drawn from the coronary sinus during controlled on-pump reperfusion: baseline, 1, 5, 15, 30, and 60 minutes of reperfusion. Immunoassays were performed for creatine kinase-muscle/brain (Cobas immunoassay CKL, ID 0-324; Roche, Bavaria, Germany) and troponin I (Immulite 1000 immunoassay; Siemens AG, Munich, Germany). Lactate was analyzed with blood gas measurements (ABL 800 flex; Drott, Wiener Neudorf, Austria).

Myocardial oxygen consumption was calculated using arterial oxygen content (CaO_2 , mL O_2 /mL) and coronary venous oxygen content (CvO_2 , mL O_2 /mL): myocardial oxygen consumption [mL O_2 /min/kg BW] = coronary flow/kg BW * ($\text{CaO}_2 - \text{CvO}_2$)/100 and for myocardial oxygen extraction: myocardial oxygen extraction [%] = ($\text{CaO}_2 - \text{CvO}_2$)/ CaO_2 * 100.

Malondialdehyde (MDA) was determined in principal according to a previously described HPLC method by Pilz and colleagues^{E7} after derivatization with 2,4-dinitrophenylhydrazine (DNPH). In brief: For alkaline hydrolysis of protein-bound MDA, 25 μL of 6 mol/L sodium hydroxide was added to 0.125 mL of EDTA plasma (1.5 mL Eppendorf tubes) and incubated at 60° (Eppendorf heater) for 30 minutes. The hydrolyzed sample was deproteinized with 62.5 μL 35% (v/v) perchloric acid. Then, 125 μL of supernatant obtained after centrifugation (14,000g; 2 minutes) was mixed with 12.5 μL of DNPH solution and incubated for 10 minutes. This reaction mixture, diluted derivatized standard solutions (0.625–10 nmol/mL), and reagent blanks were injected into the HPLC system (injection volume: 40 μL). The MDA standard was prepared by dissolving 25 μL of 1.1.3.3-tetramethoxypropane in 100 mL of bi-distilled H_2O (stock solution: 1 mmol/L). The hydrolysis was performed with 200 μL of 1.1.3.3-tetramethoxypropane stock solution in 10 mL of 1% sulfuric acid and incubation for

2 hours at room temperature.^{E8} The resulting MDA standard of 20 nmol/mL was further diluted with 1% sulfuric acid to the final concentrations. The DNPH derivatives (hydrazones) were isocratically separated on a 5- μm ODS hypersil column (150 $\times$ 4.6 mm) guarded by a 5- μm ODS hypersil column (10 $\times$ 4.6 mm; Uniguard holder) with a mobile phase consisting of a 0.2% (v/v) acetic acid solution (bidistilled water) containing 50% acetonitrile (v/v). The HPLC separations were performed with an L-2200 autosampler, an L-2130 HTA pump, and an L-2450 diode array detector (all: VWR Hitachi; Vienna, Austria). Detector signals (absorbance at 310 nm) were recorded and program EZchrom Elite (VWR) was used for data requisition and analysis.^{E9}

Quantitative Polymerase Chain Reaction (qPCR)

Analysis

RNA isolation. Tissues were homogenized in 750 μL of Qiazol with TissueRuptor (QIAGEN) for 30 seconds at full speed. RNA extraction was performed with 140 μL of chloroform, and phase separation was achieved by centrifugation for 15 minutes at 12,000g at 4°C. Then, 350 μL of the upper aqueous phase were precipitated and purified on a QIAcube liquid-handling robot using the miRNeasy Mini Kit (QIAGEN). The aqueous phase was precipitated with 525 μL of 100% ethanol and columns were washed with RWT and RPE buffer. Total RNA was eluted in a single round in 30 μL of nuclease-free water and stored at –80°C.

mRNA qPCR quantification. From isolated total RNA, cDNA was synthesized using the TATAA GrandScript cDNA Synthesis Kit (TATAA Biocenter, Goteborg, Sweden). Reverse transcription reactions were performed in 20- μL reactions with 500 ng of total RNA. For each sample, qPCRs were performed in 10- μL reactions in duplicates with 2 μL of 1:2 diluted cDNA and 8 μL of qPCR Mix consisting of 5 μL of TATAA SYBR Grandmaster Mix (TATAA Biocenter), 0.8 μL of forward and reverse primer (10 μM), and 2.2 μL of nuclease-free water. qPCR was performed on a Roche LightCycler 480 II instrument. The following thermocycling conditions were used: 30 seconds at 95°C, 45 cycles of 5 seconds at 95°C, 15 seconds at 63°C, 10 seconds at 72°C, followed by melting curve analysis. Cq values were computed using the second derivative maximum method provided with the LC480 II software. ACTB was used as a reference gene, and normalization was performed as follows: normalized $\text{Cq} = \text{Cq}^{\text{ACTB}} - \text{Cq}^{\text{GOI}}$.

The following primers were used:

Gene	Forward primer	Reverse primer
ACTB	CACGCCATCCTGCGTCTGGA	AGCACCGTGTGGCGTAGAG
TNF alpha	GGCCCAAGGACTCAGATCAT	CTGTCCCTCGGCTTTGACAT
Interleukin-6	ATGTCGAGGCTGTGCAGATT	TTGTGTTCTTCATCCACTCGT
HMGB1	GGCTGCTAAGCTGAAGGAGA	GCTGCATCAGGCTTCCCTTT
CASP3	TTGAGACGGACAGTGGGACT	CGTCCTTTGAATTCGCCAGG

ACTB, Actin beta; TNF, tumor necrosis factor; HMGB1, high mobility group box 1; CASP3, caspase 3.

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FIGURE E1. Number of direct current shocks at the beginning of reperfusion was comparable in both groups ($P = .071$). *DC*, Direct current; *STH-Pol-B*, new St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2.

FIGURE E3. Arterial malondialdehyde plasma levels were comparable in both groups during on-pump reperfusion. *STH-Pol-B*, new St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2.

FIGURE E2. To maintain systolic blood pressure >70 mm Hg, continuous noradrenaline infusion was administered. Both groups demanded a similar amount of noradrenaline support during the experiment ($P = .702$). *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2.

FIGURE E4. These representative micrographs show differences in grade of damage. On the *left*, no visible damage (grade 0: normal matrix granules) can be seen. on the *right*, moderate tissue damage (grade 2: moderate clearing of matrix, moderate swelling and partial fragmentation of cristae) can be detected. Transmission electron microscopy of samples obtained from the left anterior wall at the end of reperfusion.

FIGURE E5. Ultrastructural integrity was evaluated in samples from the left anterior wall with transmission electron microscopy in each animal from 3 randomly selected fields in a blinded manner. None of the hearts in either group showed severe to irreversible damage. In STH-Pol-B, 2 samples showed no damage, 1 slight damage, 1 slight-to-moderate damage, and 3 moderate damage. In STH2-B, 1 sample showed no damage, 1 no to slight damage, 2 slight damage, and 2 samples showed slight-to-moderate damage, indicating similar protection with both cardioplegic solutions ($P = .42$). *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2.

FIGURE E6. The anti-inflammatory miR-708-5p was significantly lower in STH-Pol-B. Consecutive assessment of proinflammatory cytokines (TNF-alpha, IL-6, and HMGB1) and apoptosis (caspase-3) related genes showed no differences between the groups. (TNF-alpha: $P = .78$; IL-6: $P = .85$; HMGB1: $P = .84$; caspase-3: $P = .09$). *STH-Pol-B*, new St Thomas' Hospital Polarizing blood cardioplegia; *TNF*, tumor necrosis factor; *IL*, interleukin; *HMGB1*, high mobility group box 1.

TABLE E1. Final concentrations of cardioplegia components after 1:2 dilution (blood: crystalloid)

Composition, mmol/L	STH-Pol-B	STH2-B
Esmolol	0.68	—
Adenosine	0.33	—
Magnesium	6.67	10.7
Sodium chloride	110	110
Potassium chloride	4	10.7
Calcium chloride	1.2	1.2

Final molar concentrations in low-dose cardioplegic solutions (STH-Pol-B and STH2-B). The basic composition of STH-Pol-B was esmolol, adenosine, and magnesium gluconate. Pig blood was mixed with the crystalloid solution (STH-Pol-B or STH2-B) immediately before administration. *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2.

TABLE E2. Baseline hemodynamics and echocardiographic data

Group	STH-Pol-B (n = 7)	STH2-B (n = 6)	P value
HR, bpm	113 ± 22	101 ± 8	.25
EF-baseline, %	58 ± 10	63 ± 5	.46
EF-R, %	63 ± 21	76 ± 11	.36
FS-baseline, %	31 ± 7	33 ± 4	.50
FS-R, %	38 ± 9	43 ± 8	.46
Systolic AP, mm Hg	72 ± 16	77 ± 13	.57
Diastolic AP, mm Hg	42 ± 11	46 ± 10	.60
MAP, mm Hg	55 ± 15	60 ± 9	.48
RAP mean, mm Hg	6 ± 5	8 ± 2	.35
Wedge, mm Hg	10 ± 3	11 ± 3	.46
Systolic LVP, mm Hg	77 ± 16	83 ± 6	.40
LVESF, mm Hg	82 ± 12	79 ± 13	.69
LVEDP, mm Hg	13 ± 3	15 ± 3	.21
CO/hw, mL/g	20.4 ± 3.3	17.0 ± 2.7	.07
SV, mL/kg/beat	188.8 ± 54.0	165.0 ± 31.4	.40
EHW, mL/kg*mm Hg	1600 ± 460	1380 ± 284	.37
CF, mL/kg/min	137 ± 109	131 ± 68	.91
Hct, %	6.9 ± 2.2	7.2 ± 1.5	.81
dp/dt _{max} , mm Hg/s	1387 ± 474	1038 ± 583	.26
dp/dt _{min} , mm Hg/s	−1666 ± 1156	−836 ± 429	.13

Baseline hemodynamic values were recorded before the initiation of cardiopulmonary bypass. Values were comparable in both groups. Values are given in mean ± standard deviation. *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2; *HR*, heart rate; *EF*, ejection fraction; *R*, reperfusion; *FS*, fractional shortening; *AP*, arterial pressure; *MAP*, mean arterial pressure; *RAP*, right atrial pressure; *LVP*, left ventricular pressure; *LVESF*, left ventricular end-systolic pressure; *LVEDP*, left ventricular end-diastolic pressure; *CO*, cardiac output; *SV*, stroke volume per kg heart weight; *EHW*, external heart work; *CF*, coronary flow per kg heart weight; *Hct*, hematocrit of cardioplegia; *dp/dt_{max}*, left ventricular contractility; *dp/dt_{min}*, left ventricular contractility.

TABLE E3. High-energy phosphates

Group	STH-Pol-B (n = 7)	STH2-B (n = 6)	P value
Hypoxanthine, nmol/mg	0.58 ± 0.25	0.93 ± 0.73	.255
AMP, nmol/mg	0.96 ± 0.34	1.15 ± 1.17	.677
ADP, nmol/mg	5.34 ± 1.09	5.85 ± 1.84	.539
ATP, nmol/mg	28.57 ± 7.88	24.54 ± 7.92	.359
PCr, nmol/mg	71.97 ± 31.07	64.60 ± 40.74	.710
Energy charge	0.89 ± 0.03	0.88 ± 0.03	.563

At the end of the experiment, tissue samples were harvested from the left ventricular free wall for energy status analysis. There were no significant differences in levels of hypoxanthine, AMP, ADP, ATP, PCr, and energy charge between groups. *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2; *AMP*, adenosine monophosphate; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate, *PCr*, phosphocreatine.

TABLE E4. TPM for the top-regulated microRNAs identified by next-generation sequencing

Group MicroRNA ID	STH-Pol-B, avg ± SD	STH2-B, avg ± SD	log2 FC	Expression level (log2 TPM)	P value
ssc-miR-708-5p	26 ± 13	45 ± 21	0.78	5.15	.019
ssc-miR-122	25 ± 35	10 ± 8	−1.37	4.07	.046
ssc-miR-369	144 ± 22	113 ± 22	−0.35	7.01	.066
ssc-miR-758	11 ± 1	8 ± 2	−0.40	3.28	.067
ssc-miR-338	32 ± 10	43 ± 15	0.41	5.24	.11
ssc-miR-323	26 ± 5	21 ± 4	−0.33	4.56	.12
ssc-miR-144	289 ± 132	429 ± 286	0.57	8.49	.12
ssc-miR-204	8 ± 2	12 ± 7	0.52	3.33	.14
ssc-miR-450b-5p	140 ± 17	119 ± 13	−0.24	7.02	.15
ssc-miR-490-5p	60 ± 3	51 ± 7	−0.23	5.80	.16
ssc-miR-503	50 ± 11	41 ± 7	−0.28	5.52	.17
ssc-miR-132	21 ± 6	17 ± 3	−0.31	4.23	.17
ssc-miR-10b	1083 ± 266	1363 ± 425	0.33	10.26	.17
ssc-miR-182	234 ± 76	181 ± 74	−0.37	7.70	.21
ssc-miR-542-3p	618 ± 88	539 ± 40	−0.20	9.18	.22
ssc-miR-450a	23 ± 3	27 ± 6	0.25	4.64	.24
ssc-miR-7134-5p	95 ± 10	112 ± 29	0.24	6.69	.24
ssc-miR-769-5p	101 ± 11	88 ± 12	−0.19	6.57	.25
ssc-miR-149	73 ± 14	63 ± 12	−0.23	6.09	.26
ssc-miR-374a-5p	1187 ± 214	1406 ± 424	0.24	10.34	.27
ssc-let-7d-5p	4011 ± 413	4514 ± 446	0.17	12.06	.28
ssc-let-7f	48,946 ± 3873	54,654 ± 3815	0.16	15.66	.28
ssc-miR-125b	754 ± 54	849 ± 126	0.17	9.65	.29
ssc-miR-340	267 ± 28	237 ± 29	−0.17	7.98	.29

Overall, 238 miRNAs were detected in all samples with a minimum abundance of 1 TPM. Table E4 provides a summary of next-generation sequencing results for the top-regulated microRNAs ($P < .3$) obtained by EdgeR, including the average (avg) expression levels (tags per million) together with standard deviation (SD), the fold change in expression between STH2-B and STH-Pol-B, as well as P value. *STH-Pol-B*, New St Thomas' Hospital Polarizing blood cardioplegia; *STH2-B*, St Thomas' Hospital blood cardioplegia No. 2; *TPM*, tags per million.

TABLE E5. Summarized endpoints

Index	time $\times$ group interaction		Main effect of group		Main effect of time		Favors
HR	F(7, 44.7) = 0.832	P = .567	F(1,10.0) = 2.652	P = .18	F(7,33.7) = 2.652	P = .027	Similar
Systolic AP	F(2, 11) = 0.650	P = .541	F(1, 9.8) = 2.778	P = .127	F(2,11.4) = 0.925	P = .424	Similar
Diastolic AP	F(2,23.6) = 2.649	P = .092	F(1,8.8) = 6.519	P = .032	F(2,17.2) = 8.638	P = .003	STH-Pol-B
MAP	F(2,22) = 2.697	P = .090	F(1,10) = 11.604	P = .007	F(2,12) = 9.937	P = .003	STH-Pol-B
RAP mean	F(2,10.3) = 2.296	P = .149	F(1,8.6) = 1.238	P = .296	F(2,11.4) = 1.346	P = .299	Similar
Systolic LVP	F(2,11.0) = 0.522	P = .607	F(1,10) = 25.609	P = .000	F(2,12.0) = 3.088	P = .083	STH-Pol-B
SV	F(2,8.1) = 0.141	P = .870	F(1,8) = 9.471	P = .015	F(2,20) = 10.953	P = .001	STH-Pol-B
CO	F(2,10.4) = 1.173	P = .347	F(1,9) = 1.310	P = .282	F(2,13.6) = 8.091	P = .005	Similar
EHW	F(2,9.3) = 0.119	P = .889	F(1,7.9) = 10.442	P = .012	F(2,10.3) = 4.292	P = .044	STH-Pol-B
LVESP	F(2,11.0) = 1.578	P = .250	F(1,10.0) = 10.151	P = .010	F(2,12.0) = 3.448	P = .066	STH-Pol-B
LVEDP	F(2,22) = 2.959	P = .073	F(1,10) = 0.735	P = .411	F(2,12) = 0.796	P = .474	Similar
CF	F(7,45.8) = 0.692	P = .678	F(1,9.6) = 6.467	P = .030	F(7,29.6) = 2.031	P = .084	STH-Pol-B
Wedge	F(2,11.2) = 1.834	P = .205	F(1,9.4) = 8.996	P = .014	F(2,12.5) = 0.582	P = .573	STH-Pol-B
dp/dt _{max}	F(2,22) = 0.852	P = .440	F(1,10) = 6.733	P = .027	F(2,24) = 2.380	P = .114	STH-Pol-B
Noradrenaline	F(7,16.4) = 0.951	P = .496	F(1,10.0) = 0.155	P = .702	F(7,18.3) = 3.354	P = .018	Similar
Arterial CK-MB	F(7,14.9) = 1.323	P = .310	F(1,9.9) = 0.1288	P = .283	F(7,14.8) = 26.050	P = .000	Similar
Coronary CK-MB	F(4,11.8) = 0.393	P = .810	F(1,9) = 4.693	P = .058	F(4,44) = 26.965	P = .000	Similar
Arterial troponin-I	F(7,21.4) = 2.061	P = .093	F(1,10) = 0.415	P = .534	F(7,84) = 135.786	P = .000	Similar
Malondialdehyde	F(4,10.2) = 1.147	P = .389	F(1,4.3) = 0.159	P = .709	F(4,12.1) = 0.275	P = .888	Similar
Arterial lactate	F(7,17.4) = 2.312	P = .074	F(1,8.5) = 0.493	P = .501	F(7,18.2) = 1.191	P = .356	Similar
Coronary lactate	F(4,25.5) = 4.871	P = .005	F(1,10.1) = 0.300	P = .596	F(4,25.5) = 2.202	P = .097	Similar
Coronary pH	F(4,21.2) = 5.640	P = .003	F(1,9.227) = 7.572	P = .022	F(4,21.2) = 1.905	P = .147	Similar
Coronary venous oxygen content	F(4,15.23) = 1.259	P = .328	F(1,10) = 4.160	P = .069	F(4,48) = 2.561	P = .050	Similar

HR, Heart rate; AP, arterial pressure; *STH-Pol-B*, new St Thomas' Hospital Polarizing blood cardioplegia; MAP, mean arterial pressure; RAP, right atrial pressure; LVP, left ventricular pressure; SV, stroke volume per kg heart weight; CO, cardiac output; EHW, external heart work; LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; CF, coronary flow; dp/dt_{max}, left ventricular contractility; CK-MB, creatine kinase-muscle/brain.