Carvedilol-Enriched Cold Oxygenated Blood Cardioplegia Improves Left Ventricular Diastolic Function After Weaning From Cardiopulmonary Bypass

Geir Olav Dahle, MD,*† Pirjo-Riitta Salminen, MD, PhD,*† Christian Arvei Moen, MD, PhD,† Finn Eliassen, CCP,* Else Nygreen, CCP,* Ville Kytö, MD, PhD,‡§ Pekka Saukko, MD, PhD,¶ Rune Haaverstad, MD, PhD,*† Knut Matre, MSc, PhD,† and Ketil Grong, MD, PhD†

Objectives: **To investigate whether adding carvedilol, a nonselective β- and selective α_1-receptor blocking agent with antioxidant properties, to oxygenated blood cardioplegia improves myocardial function after weaning from bypass.**

Design: **A randomized controlled study.**

Setting: **A university laboratory.**

Participants: **Twenty anesthetized pigs, Norwegian Landrace.**

Interventions: **On cardiopulmonary bypass, cardiac arrest was induced with cold (12°C), oxygenated blood cardioplegia, enriched with carvedilol or vehicle, and repeated every 20 minutes. After 100 minutes, the heart was reperfused and weaned.**

Measurements and Main Results: **Left ventricular function was evaluated with pressure-volume loops, local myocardial systolic strain, and strain rate from Speckle tracking analysis and multilayer short-axis tissue Doppler Imaging. In the carvedilol group, the load-independent logarithmic end-diastolic pressure volume relationship, β, decreased from 1 to 3 hours of reperfusion and was low, 0.028 ± 0.004 v 0.042 ± 0.007 ($p < 0.05$) in controls at 3 hours, demonstrating improved left ventricular compliance. The diastolic relaxation constant τ was decreased, 28.9 ± 0.6 ms v 34.6 ± 1.3 ms ($p_g < 0.035$), and dP/dt_{min} was more negative, −1,462 ± 145 mmHg/s v −1,105 ± 105 mmHg/s ($p_g = 0.024$), for carvedilol v control group. The systolic variables, preload recruitable stroke work and end-systolic pressure-volume relationship, did not differ between groups, neither did left ventricular systolic strain and strain rate. Myocardial oxidative stress, measured as tissue levels of malondialdehyde, was reduced by carvedilol, 0.19 ± 0.01 compared to 0.24 ± 0.01 nmol/mg ($p = 0.004$) in controls.**

Conclusions: **Carvedilol added to blood cardioplegia improved diastolic cardiac function and reduced oxidative stress during the first 3 hours after reperfusion in a porcine model, with 100 minutes of cardioplegic arrest.**

KEY WORDS: carvedilol, cardiac function, cardioplegia, cardiopulmonary bypass, oxidative stress

TRANSITORY POSTOPERATIVE left ventricular dysfunction, lasting for hours or days, is observed after cardiopulmonary bypass (CPB) and cardioplegic arrest. This dysfunction is related to factors like ischemia/reperfusion injury, apoptosis, adrenergic-receptor desensitization, and potassium load. Beta-adrenergic receptor blocking agents reduce both ischemic and reperfusion injury in the myocardium.[1-4] Perioperative beta-blockers are also beneficial in cardiac surgery.[5,6] In experimental protocols, both pretreatment with the short-acting β-blocker esmolol or addition of esmolol in cold, intermittent, oxygenated blood cardioplegia improves myocardial contractility in the early hours after CPB and cardiac arrest.[7,8] The occurrence of oxidative stress is a major component in both lethal reperfusion injury and in recruitment and activations of neutrophil granulocytes as part of the inflammatory response to CPB.[9] Carvedilol, a non-selective beta- and alpha$_1$-receptor blocker with powerful antioxidative properties, is therefore of particular interest as a cardioprotective agent.[10] Several studies have shown carvedilol to be superior to traditional beta-blockers in preserving cardiac function and reducing ischemic and lethal reperfusion injury in the context of coronary occlusion and reperfusion.[2,3,11] One animal model and one *in vitro* study on human atrial myocytes in a perfusion chamber have demonstrated that carvedilol was acting as an antiapoptotic agent in the context of cardiopulmonary bypass and cardioplegic arrest.[12,13] In these studies, however, the description of myocardial performance after reperfusion was sparse.

Hyperkalemic, cold, intermittent oxygenated blood cardioplegia is considered to be the gold standard for myocardial protection during cardiac surgery.[14] When using intermittent oxygenated blood cardioplegia, cardioplegic arrest is the starting point of an anticipated ischemic period of the myocardium also including several episodes of reperfusion and reoxygenation. Intracoronary administration via the cardioplegic solution allows the myocardium to be exposed to a relatively high concentration of carvedilol during the extracorporal circulation while avoiding large systemic doses that may cause severe hemodynamic depression after weaning from CPB. In this study the authors hypothesized that carvedilol added in the cardioplegic concentrate would reduce lethal reperfusion injury and improve cardiac performance during the first hours after weaning from CPB with 100 minutes of cardioplegic arrest by repeated cold oxygenated blood cardioplegia.

METHODS

Animals and Anesthesia

Twenty-six pigs weighing 43 ± 3 kg (standard deviation) were used in this study. After at least 7 days of acclimatization

*From the *Section of Cardiothoracic Surgery, Department of Heart Disease, Haukeland University Hospital, Bergen, Norway; †Department of Clinical Science, University of Bergen, Bergen, Norway; ‡Heart Center, Turku University Hospital, University of Turku, Turku, Finland; §Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; and ¶Department of Pathology and Forensic Medicine, University of Turku, Turku, Finland.*

Address reprint requests to Geir Olav Dahle, MD, Department of Clinical Science, University of Bergen, Haukeland University Hospital, NO-5021 Bergen, Norway. E-mail: geir.olav.dahle@helse-bergen.no

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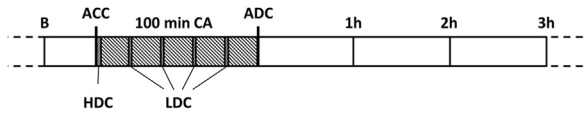

Fig 1. Schematic timeline for experiments. B, baseline; ACC and ADC, aortic cross-clamping and declamping; CA, cardioplegic arrest; HDC and LDC, high- and low-dose cardioplegia; 1h, 2h, and 3h, time of measurements at one, two, and three hours after aortic declamping.

in the animal facility, the pigs were fasted overnight but had free access to water. All procedures were performed in accordance with international guidelines described in European Communities Council Directive of 2010 (63/EU). The experimental protocol was approved by the Norwegian State Commission for Laboratory Animals (project No. 20113923).

Ketamine (20 mg/kg), diazepam (10 mg), and atropine (1 mg) injected intramuscularly in the dorsal region of the neck served as premedication. Animals were ventilated with isoflurane, 3% in oxygen, for a brief period while 2 ear veins were cannulated. Intravenous anesthesia then was initiated with loading doses and continuous infusions with pentobarbital (15 mg/kg and 4 mg/kg/h), midazolam (0.3 mg/kg and 0.3 mg/kg/h), fentanyl (0.02 mg/kg and 0.02 mg/kg/h) and vecuronium (0.6 mg/kg and 0.3 mg/kg/h). A tracheotomy was performed and mechanical ventilation commenced (Julian, Dräger, Lübeck, Germany) with fixed tidal volumes set at 11 mL/kg. A mixture of 57% N_2O and oxygen was used. Respiratory rate was adjusted to maintain end-tidal CO_2 between 5.0 and 5.7 kPa (38 and 43 mmHg). This anesthetic protocol has been evaluated thoroughly and found to be hemodynamically stable for at least 7 hours.[15,16] At the end of the experiments, the animals, still under general anesthesia, were euthanized with saturated potassium chloride injected into the left atrium.

Surgical Instrumentation

The right femoral artery and vein were cannulated for blood sampling. A suprapubic catheter was placed into the urinary bladder and urinary output measured. After a midline sternotomy and pericardiotomy, a 6F catheter was placed in the left atrium for microsphere injections, and sutures for bypass cannulation were prepared. A loose snare was placed around the inferior vena cava allowing acute dynamic preload reductions. A pressure-tip catheter (MPC-500, Millar Corp., Houston, TX) was placed in the aortic arch via the left internal thoracic artery. A Swan-Ganz catheter (177HF75-7.5F, Edward Lifesciences Inc., Irvine, CA) was advanced through the right ventricle from the left internal thoracic vein to obtain continuous cardiac output, right ventricular end-diastolic volume (RV-EDV), and right ventricular ejection fraction (RV-EF) as well as central venous and pulmonary artery pressures. The catheter was connected to pressure transducers (TruWave®, Edward Lifesciences) and a continuous cardiac output computer (Vigilance II, Edward Lifesciences). Hemodynamic measurements were digitized and continuously recorded with a signal conditioning unit (ACQ-7700, Data Sciences International, St. Paul, MN). Finally a pressure-conductance catheter (CDLeycom, Hengelo, The Netherlands) was placed through the left ventricle from the apex and connected to a signal conditioner unit (Sigma-M, CDLeycom). Correct placement was confirmed with echocardiography (Vivid E9, GE Vingmed Ultrasound, Horten, Norway). After instrumentation, animals were left to stabilize for 15 minutes before baseline measurements.

Cardiopulmonary Bypass

Cannulation for CPB was done through the brachiocephalic artery and the right atrial appendage with an 18F arterial cannula (Medtronic Inc., Minneapolis, MN) and a 29F cavoatrial 3-stage cannula (Medtronic Inc.). The priming volume (1,200 mL Ringer's acetate) and blood were mixed at full CPB flow (90 mL/kg/min) for a few minutes before aortic cross-clamping. A left ventricle venting catheter (E061 17-Fr, Edwards Lifesciences, Inc.) was introduced via the left atrial appendage. At a flow rate of 7% of full CPB flow (6.3 mL/kg/min), hearts were perfused with the "high-dose" oxygenated cold blood cardioplegia through the aortic root for 3 minutes (Fig 1, Table 1). After this, new cardioplegic "low-dose" perfusions were repeated for 2 minutes every 20 minutes. Active cooling allowed core temperature to drift towards 35°C, and when reached or after 20 minutes of cardioplegic arrest, CPB flow was reduced to 72 mL/kg/min. After 80 minutes, rewarming to baseline temperature commenced and CPB flow was reset to 90 mL/kg/min. Arterial blood gases were drawn just before, after 50 minutes, and after 98 minutes of aortic cross-clamping. The aorta was declamped after 100 minutes, and animals were weaned from CPB and decannulated within 20 minutes. The only antiarrhythmic intervention allowed in this protocol was electroconversion from ventricular fibrillation if needed.

Design

Animals were block-randomized into a carvedilol group or a control group. In the carvedilol group, the cardioplegic concentrate (1,000 mL) was prepared with 8 mg-carvedilol dissolved in a vehicle of 250 μL of dimethylformamide and 50 μL of HCl on the day of the experiment. The mixture then was diluted carefully with the cardioplegic concentrate under constant stirring to avoid any precipitation. In the control group, the cardioplegic electrolyte concentrate was prepared in the same manner but enriched with vehicle only. Evaluation of cardiac function, tissue blood flow with microspheres, and blood sampling was performed at baseline and then every hour

Table 1. Calculated Concentrations in Oxygenated Blood Cardioplegia

	High Dose	Low Dose
K^+ (mM)	22	14
Mg^{2+} (mM)	16	9
Cl^- (mM)	134	120
Procainhydrocloride (mm)	0.8	0.4
DMF (μM)	0.6	0.3
Carvedilol* (μM)	4	2.2

Abbreviation: DMF, dimethylformamide.

*In intervention group only.

after aortic declamping for 3 hours (Fig 1). Tissue samples were harvested at the end of experiments.

Pressure-Volume Loops

When evaluating cardiac function, 8 to 12 stable heartbeats were recorded and averaged to evaluate left ventricular pressures and volumes. Following this, 5 to 10 (mean 7.8) beats during an acute dynamic preload reduction, obtained by inferior vena cava constriction, were recorded with the pressure-conductance catheter to assess load-independent left ventricular systolic and diastolic variables. Three separate calibration sequences with 5 mL of 10% saline injected into the pulmonary artery then were recorded. All measurements were done during respirator shut-off, and animals were allowed to stabilize to initial hemodynamic values between recordings. Unfiltered data were exported to an in-house-developed software coded in Matlab (MathWorks Inc., Natick, MA) for further analysis. Paradoxical segmental conductance signals, indicating aortic positioning, were excluded from the total conductance signal. Parallel conductance was calculated from the recordings during hypertonic saline injections, whereas stroke volume from the continuous cardiac output computer served as alpha correction.[17] Left ventricular volumes were normalized for body surface area.[18] The load-independent measures of contractility, preload recruitable stroke work (PRSW), and end-systolic pressure-volume relationship (ESPVR) and diastolic compliance (β), calculated as the exponential fit of the end-diastolic pressure volume relationship (EDPVR) ($p = C \cdot e^{\beta V}$),[19] were based on a minimum of 5 consecutive beats during preload reduction. The median correlation coefficients (interquartile ranges) were 0.998 (0.999; 0.996) for PRSW, 0.993 (0.997; 0.984) for ESPVR and 0.992 (0.996; 0.974) for β. The change in heart rate between the first and last cycle was 1.4 ± 3.2 (standard deviation) beats/min for all 80 runs. The isovolumic relaxation constant was calculated by the left ventricular pressure waveform assuming a non-asymptotic decay.[20]

Echocardiography

After each microsphere injection and pressure-volume loop recording, echocardiography was performed with a Vivid E9 scanner (GE Vingmed Ultrasound). A soft silicon pad (4 × 4 × 3 cm) serving as an offset was placed between the epicardium and the sector probe (6S cardiac probe, GE Vingmed Ultrasound). The respirator was disconnected during recordings. Cine-loops of short-axis B-mode view at the equator were recorded for left ventricular radial and circumferential strain and strain rate using speckle tracking analysis (STE). With the same probe position recordings of the anterior wall optimized for multilayer radial tissue Doppler imaging (TDI) were obtained. The probe then was relocated to the apical long-axis view for pulsed-wave Doppler velocity recording from the mitral and aortic valves for timing purposes. Still in long-axis view, 4-chamber B-mode cine-loops of the left ventricle were obtained for STE analysis. All analyses were carried out using EchoPac BT12 (GE Vingmed Ultrasound) with the operator being unaware of the data randomization. End-diastole was defined as the beginning of the first deflection of the QRS complex on electrocardiogram, whereas the end-systole was defined as the time of aortic valve closure on corresponding pulsed-wave Doppler spectral recordings. For TDI analysis, strain length was set to 2 mm, and 3 regions of interest measuring 2 × 6 mm were tracked evenly through the anterior left ventricular wall representing the subendocardial, midmyocardial, and subepicardial part of the wall.[21]

Tissue and Blood Samples

Immediately after euthanasia, the heart was removed and samples from the left ventricle, divided into subendocardial, midmyocardial, and subepicardial layers, and from the right ventricle were snap-frozen in liquid nitrogen and stored at −80°C. Corresponding tissue samples were placed in paraformaldehyde, washed in ethanol, embedded in paraffin, and sectioned for histology. Also, 2 more sets of corresponding tissue were harvested and weighed for tissue water content and quantification of tissue blood flow rate.

Oxidative stress was studied by lipid peroxidation assessed by measuring malondialdehyde (MDA) in 10-mg tissue samples with a fluorometric kit (K739-100, BioVision Inc., Milpitas, CA). Likewise, a Caspase-3 Colorimetric Assay kit (K105-400, BioVision Inc.) was used to quantify caspase-3 activity in tissue samples of 400 μg of total protein as a marker of initiation of apoptosis. For both kits, triplet samples were used and tissue was homogenized, lysed, and measured according to the manufacturer's instructions.

Cardiomyocyte apoptosis was studied with the terminal transferase-mediated DNA nick end labeling (TUNEL) assay.[22] In brief, paraffin-embedded myocardial sections (4 μm) were heated in a sodium citrate solution and digested with proteinase-K to expose DNA. Strand breaks of DNA were then labeled using terminal transferase with digoxigenin conjugated dideoxyuridine triphosphate and visualized with alkaline phosphatase immunohistochemistry. Assay was standardized using the serial section of each sample treated with DNaseI as positive control for apoptosis. Percentages of TUNEL-positive nuclei were calculated using microscopy (×250 magnification) with an ocular grid.

The myocardial tissue and corresponding reference blood samples collected for flow rate estimation were weighed, hydrolyzed, and filtered. Fluorescent dye extracted from microspheres on the filter was quantified by flurospectrophotometry (RF-5301PC, Shimadzu, Kyoto, Japan) and regional tissue blood flow rate calculated.[23] Tissue water content, presented as fraction of wet-weight, was calculated as the difference between wet-weight measured immediately after harvest and after 3 weeks of drying at 60°C.

Troponin-T was analyzed according to the hospital clinical routine (Troponin-T hs®, Roche Diagnostics, Mannheim, Germany). All arterial blood gas samples were analyzed according to α-stat strategy (Pico 50, Radiometer Medical, Brønshøj, Denmark).

Statistics

Data were analyzed by using SPSS *v*23 (SPSS Inc., Chicago, IL) and values presented as mean ± standard error of the mean or median (75-percentile; 25-percentile), unless

otherwise noted. Baseline variables demonstrating normal distribution were compared by 2-sample Student t-tests. The 2-sample Wilcoxon-Mann-Whitney test on ranks was used if the Kolmogorov-Smirnov test or the Levene equal variance tests were significant when comparing baseline variables and tissue Caspase-3 activity in wall layers. Variables obtained during and after CPB were analyzed separately by 2-way analyses of variance for repeated measurement (RM-ANOVA) with time as within factor (p_w) and carvedilol or control as grouping factor (p_g) and *post hoc* Bonferroni contrasts between individual group means. A similar analysis was used for related samples in wall layers. If Mauchly's test of sphericity was significant ($p < 0.05$), the Greenhouse-Geisser adjustment of degrees of freedom was selected for the evaluation of main effects. If a significant interaction ($p_i < 0.10$) effect was found, new ANOVAs for simple main effects were performed with adjustment of degrees of freedom. Cell means finally were compared with Neumann-Keuls multiple contrast tests when justified by the preceding ANOVA. A p value < 0.05 was considered as statistically significant.

Table 2. Baseline Variables Before Cardioplegic Arrest

Variable	Carvedilol (n = 10)	Control (n = 10)	p*
HR (beats/min)	85 ± 4	79 ± 3	0.24
LV-ESP (mmHg)	104 ± 6	95 ± 4	0.19
LV-ESV$_i$ (mL/m^2)	28 ± 2	29 ± 2	0.76
LV-EDP (mmHg)	9.4 ± 0.7	10.3 ± 0.7	0.35
LV-EDV$_i$ (mL/m^2)	72 (77; 68)	79 (85; 73)	0.21
SV$_i$ (mL/m^2)	44 ± 3	50 ± 2	0.056
CI (L/min/m^2)	3.6 ± 0.2	4.0 ± 0.2	0.28
LV-EF (%)	61 ± 3	64 ± 2	0.36
LV-SW$_i$ (mmHg · mL/m^2)	3,917 ± 314	4,184 ± 225	0.50
RV-EDV$_i$ (mL/m^2)	140 ± 8	147 ± 7	0.54
RV-EF (%)	25 ± 1	25 ± 1	0.91
STE-CircS (%)	−15.8 ± 1.5	−18.8 ± 0.6	0.08
STE-LongS (%)	−12.1 ± 0.9	−13.7 ± 0.9	0.23
STE-RadS (%)	27.2 (48.6; 24.9)	28.2 (36.6; 26.6)	0.85
STE-CircSr (s^{-1})	−1.15 ± 0.10	−1.22 ± 0.05	0.50
STE-LongSr (s^{-1})	−1.05 ± 0.04	−1.03 ± 0.03	0.75
STE-RadSr (s^{-1})	2.27 ± 0.23	1.98 ± 0.10	0.25
LV-blood flow (mL/min/g)†	0.78 ± 0.03	0.75 ± 0.05	0.58
RV-blood flow (mL/min/g)†	0.56 ± 0.03	0.61 ± 0.05	0.33
MAP (mmHg)	95 ± 5	86 ± 4	0.18
PAP (mmHg)	17.0 ± 1.6	17.1 ± 0.9	0.94
CVP (mmHg)	4.8 ± 0.7	5.5 ± 0.6	0.42
LV-PFR/EDV (s^{-1})	3.9 ± 0.2	4.0 ± 0.3	0.88
SVR$_i$ (dyn · s/cm^5 · m^2)	2,025 ± 134	1,646 ± 106	0.040

NOTE. Values are mean ± SEM or median (75-percentile; 25-percentile).

Abbreviations: CI, cardiac index; Circ, circumferential direction; CVP, mean central venous pressure; EDP, end-diastolic pressure; EDV, end-diastolic volume; EF, ejection fraction; ESP, end-systolic pressure; ESV, end-systolic volume; HR, heart rate; i, value indexed for body surface area; Long, longitudinal direction; LV, left ventricle; MAP, mean arterial pressure; PAP, mean pulmonary artery pressure; PFR, peak filling rate; Rad, radial direction; RV, right ventricle; STE, left ventricular Speckle Tracking Echocardiography; SV, stroke volume; SW, stroke work; S, peak systolic strain; Sr, peak systolic strain rate; SVR, systemic vascular resistance.

*p values from 2-sample t-tests or Mann-Whitney rank sum tests.

†n = 9 in Carvedilol group.

RESULTS

A total of 6 animals were excluded for non-technical reasons. In the control group, 3 pigs developed severe pulmonary hypertension and arterial hypoxia shortly after weaning from CPB. In 1 of these animals, a persistent foramen ovale was found. One animal in the control group developed severe tachyarrhythmia, making evaluation of cardiac function with pressure-volume loops meaningless. One animal in the control group did not regain spontaneous rhythm and could therefore not be weaned from CPB. One animal in the carvedilol group developed cardiac fibrillation after weaning and severe cardiac failure after multiple attempts of electrocardioversion. Excluded animals were replaced in the subsequent experiment. Results are given for 10 animals in each group.

Except for a higher systemic vascular resistance (SVR_i) in the carvedilol compared to the control group, there were no significant differences with regard to baseline variables describing left and right ventricular function, hemodynamics, myocardial blood flow, arterial blood gas analysis, and any other variable evaluated (Table 2). Mean aortic pressure (MAP) differed between groups 2 minutes before aortic clamping (Table 3). All other measurements obtained just before aortic cross-clamping, during cardioplegic arrest, and just prior to declamping did not differ. The time from declamping until spontaneous heart rhythm averaged 189 ± 25 s (n = 20), and the time to cardiac pulsations averaged 318 ± 31 s with no significant difference between groups.

Heart rate and MAP did not differ between groups after aortic declamping ($p_g = 0.22$ and $p_g = 0.18$). For both groups, heart rate increased ($p_w = 0.001$) and MAP decreased ($p_w < 0.001$) from 30 minutes to 3 hours after declamping (Fig 2). There was an early increased cardiac index in both groups from 30 to 40 minutes followed by a slight decrease over time ($p_w < 0.047$) and with no difference between groups ($p_g = 0.28$). Parallel to this, stroke volume decreased in both groups (Table 4). A similar decrease was observed for left ventricular end-diastolic volume ($LV\text{-}EDV_i$), stroke work ($LV\text{-}SW_i$) and $RV\text{-}EDV_i$. Mean pulmonary artery pressure, left ventricular end-systolic pressure (LV-ESP), and SVR_i decreased over time. LV-ESP was elevated in the carvedilol compared to the control group ($p_g = 0.023$).

A significant and gradual decrease was seen in PRSW and in peak positive of the first derivative of left ventricular pressure (dP/dt_{max}) during reperfusion in both groups (Fig 3). Two hours after reperfusion, dP/dt_{max} was higher in carvedilol-treated compared to controls, but no significant difference was detectable after 3 hours. Circumferential, longitudinal, and radial peak systolic STE strain decreased during the three hours after aortic declamping with no significant differences between groups (Table 4). Peak systolic STE strain rate remained unaltered. Measured by TDI, the peak systolic radial strain and strain rate in the left ventricular anterior wall in 3-wall layers showed the same patterns as STE-derived data (Supplementary Table 1).

Table 3. Mean Arterial Pressure, Temperature, Hemoglobin, and Arterial Blood Gases During Cardiopulmonary Bypass and Aortic Clamp in Two Groups of Pigs With 100 Minutes of Cardioplegic Arrest

				RM-ANOVA Statistics		
Variable	2 Min Before X-clamp	50 Min X-clamp	98 Min X-clamp	p_w	p_g	p_i
MAP (mmHg)						
Carvedilol	56 ± 3*	47 ± 2*	56 ± 4‡	0.017	0.80	0.041
Control	47 ± 3	51 ± 4	57 ± 5†			
$Temp_{rect}$ (°C)						
Carvedilol	38.7 ± 0.2	34.5 ± 0.1	37.7 ± 0.2	<0.001	0.58	0.61
Control	38.4 ± 0.2	34.5 ± 0.1	37.6 ± 0.2			
Hb (g/dL)						
Carvedilol	5.7 ± 0.2	6.5 ± 0.2	6.9 ± 0.2	<0.001	0.41	0.46
Control	5.9 ± 0.2	6.9 ± 0.2	7.0 ± 0.2			
pH						
Carvedilol	7.40 ± 0.01	7.42 ± 0.01	7.47 ± 0.01	<0.001	0.059	0.47
Control	7.38 ± 0.01	7.39 ± 0.01	7.43 ± 0.02			
pO_2 (kPa)						
Carvedilol	16.8 ± 2.0	22.8 ± 2.7	15.9 ± 1.9	0.088	0.28	0.15
Control	18.8 ± 3.0	16.4 ± 1.9	13.9 ± 0.8			
pCO_2 (kPa)						
Carvedilol	5.6 ± 0.2	5.7 ± 0.1	5.0 ± 0.2	<0.001	0.12	0.70
Control	5.8 ± 0.1	6.1 ± 0.2	5.4 ± 0.3			
HCO_3^- (mmol/L)						
Carvedilol	25.5 ± 0.6	27.0 ± 0.6	27.7 ± 0.7	<0.001	0.25	0.35
Control	25.1 ± 0.5	26.2 ± 0.3	26.5 ± 0.3			
s-K^+ (mmol/L)						
Carvedilol	4.4 ± 0.1	4.8 ± 0.1	5.6 ± 0.2	<0.001	0.23	0.61
Control	4.3 ± 0.1	4.7 ± 0.1	5.4 ± 0.2			
s-Na^+ (mmol/L)						
Carvedilol	141 ± 1	142 ± 1	140 ± 1	0.20	0.45	0.38
Control	140 ± 1	141 ± 1	140 ± 1			

NOTE. Values are mean ± SEM, n = 10.

Abbreviations: MAP, mean aortic pressure; p_g, p value between groups from 2-way RM-ANOVA; p_i, p value for interaction from 2-way RM-ANOVA; p_w, p value within-subjects from 2-way RM-ANOVA; RM-ANOVA, analyses of variance for repeated measurement; s, serum levels; $Temp_{rect}$, rectal temperature.

*Significantly different from control at 2 min before X-clamp.

†Significantly different from 2 min before X-clamp.

‡Significantly different from at 50 min of X-clamp

Peak negative left ventricular pressure decay rate (dP/dt_{min}) gradually decreased (less negative) from 60 to 180 minutes after declamping in both groups (Fig 3). Furthermore, dP/dt_{min} was more negative in carvedilol-treated animals compared to controls during the observation period. The end-diastolic compliance, β, decreased significantly in the carvedilol group with a tendency to increase in the control group ($p_i = 0.028$). At 180 minutes after declamping β was significantly decreased in carvedilol-treated animals compared to controls. In the carvedilol group the left ventricular isovolumic relaxation constant, τ, was significantly reduced compared to controls after declamping.

Biopsies from the left ventricle demonstrated a reduction of tissue MDA in carvedilol-treated animals irrespective of wall layer ($p_g = 0.004$) (Fig 4). An increased number of TUNEL-positive nuclei were found in the subendocardium compared to the midmyocardium and the subepicardium ($p_w = 0.004$) but with no significant difference between treatment groups ($p_g = 0.93$). Carvedilol-treated animals displayed much variation in measurements of tissue caspase-3 activity, resulting in skewed distribution of data and no statistical differences between groups in any wall layer. Troponin-T values in serum did not differ between groups and averaged 46 ± 5 ng/L at baseline and 356 ± 24 ng/L 180 minutes after declamping (n = 20).

DISCUSSION

In this experimental model, carvedilol, added to repeated, cold, oxygenated blood, cardioplegia enhanced left ventricular diastolic function during the first 3 hours after declamping following 100 minutes of cardioplegic arrest. A more negative dP/dt_{min} and a shortening of the isovolumic relaxation constant τ in the carvedilol group compared to the control group indicated more efficient isovolumic relaxation (Fig 3). Furthermore, the end-diastolic compliance was increased significantly in the same group 3 hours after declamping demonstrated by the reduced slope of the logarithmic EDPVR, β. There was a significant increase in LV-ESP in the carvedilol group after aortic declamping. Small afterload increases can shorten τ and accelerate left ventricular pressure decay (dP/dt_{min}); a more pronounced afterload increase will have the opposite effect.[24] On the other hand, since β is a load-

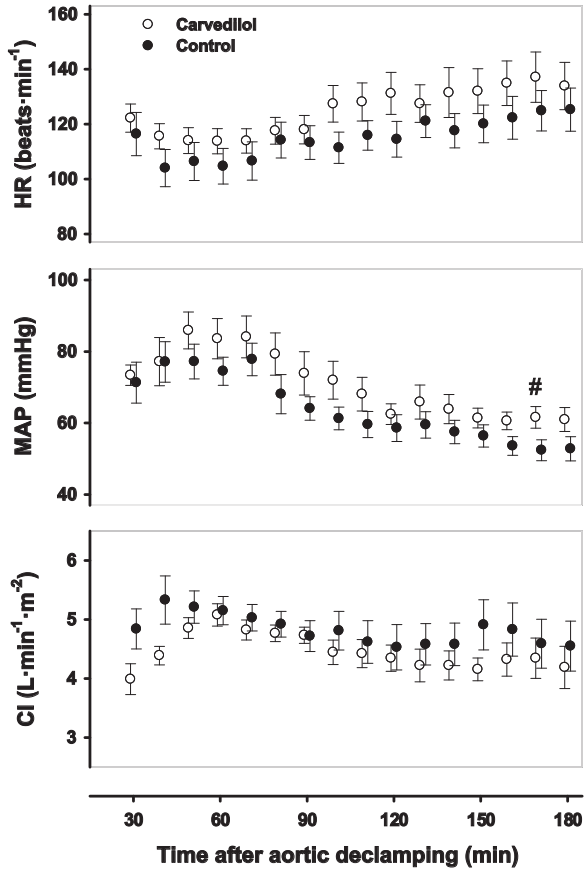

Fig 2. Trend curves for heart rate (HR), mean arterial pressure (MAP), and cardiac index (CI) from 30 to 180 minutes after aortic declamping. For CI, n = 9 in the carvedilol group due to technical failure. Values are mean ± SEM. # = statistical significant difference between groups at 170 minutes.

independent variable describing diastolic compliance, the improved diastolic function caused by carvedilol is most likely a result of improved lusitropy.

The improvement in diastolic function could be explained by increased beta-adrenergic stimulation (ie, by less desensitization) as adrenergic stimuli via cAMP accelerates the cytosolic calcium removal. However, increased adrenergic stimulation also should be accompanied by a concomitant increase in systolic variables. This was demonstrated in a similar model using the ultra-short-acting beta-blocker esmolol as an additive to the cardioplegic solution.[8] In the present study, no convincing improvement in left ventricular systolic function was demonstrated (Fig 3). More likely, the antioxidant properties of carvedilol caused the enhanced diastolic function. During ischemia, the elevated level of reactive oxygen species (ROS) is associated with cytosolic calcium overload. In the present study, the lower MDA levels in the carvedilol group indicated less oxidative stress over time. Carvedilol is a scavenger, reducing oxidative stress, and it has been shown that carvedilol prevents contractures after ischemia in reperfusion models resulting in both better isovolumic relaxation and end-diastolic compliance.[4,11,25]

In the setting of CPB and cardioplegic arrest, adrenergic blocking agents may preserve postoperative function by blocking the passing catecholamine surge, thus reducing desensitization of adrenergic receptors. This is likely to be the mechanism behind the better-preserved contractile function after CPB demonstrated for esmolol.[8] PRSW, dP/dt_{max} and echo-derived strain rate increased with esmolol treatment. In the present study, a significant difference between groups for dP/dt_{max} 2 hours after declamping was found but no differences in ESPVR or PRSW. Furthermore, there was no difference between groups in radial strain rate, also considered to be a relatively load-independent contractility variable.[26] Thus, the present study did not convincingly demonstrate an effect on inotropy or on chronotropy by carvedilol. Prevailing adrenergic receptor blockade by carvedilol after reperfusion may prevent the adrenergic receptors from responding to endogenous catecholamines in spite of being more sensitized. On the other hand, the anticipated systemic plasma concentration of carvedilol during and after cardioplegic arrest is low compared to concentrations shown to have only minimal hemodynamic suppressive effects.[3]

Carvedilol prevents beta-adrenergic receptor desensitization to some extent,[27] but in contrast to traditional beta-blockers, long-term use of carvedilol does not upregulate beta-receptor densities.[28] This has been explained by sophisticated models demonstrating that carvedilol acts as a biased agonist at an intracellular level.[29,30] The binding of carvedilol to the adrenergic receptor will not stimulate the G-protein to activate cAMP signaling via adenylyl cyclase. In contrast to traditional beta-blockers like esmolol, however, carvedilol will stimulate G-protein-receptor signaling pathway involving beta-arresting, which is involved in the decoupling and later internalization of the adrenergic receptors. Carvedilol may thus be less suited for protection from the acute beta-adrenergic receptor desensitization induced by CPB and cardioplegic arrest.

$Alpha_1$-adrenergic receptors also are known to desensitize early during acute endogenous surges of cathecholamines. Alpha-andrenergic antagonists should therefore have the potential to prevent the acute decoupling during events like CPB and cardioplegia.[31,32] To the authors' knowledge, the effect of carvedilol on acute $alpha_1$-receptor desensitization has not been studied. In the present study, an increase in LV-ESP in carvedilol-treated animals indicated elevated afterload (Table 4). Even if systemic concentrations are low during cardioplegic arrest, carvedilol might alleviate receptor desensitization by blocking vascular $alpha_1$-adrenergic receptors during CPB. At baseline there seems to be an elevated afterload in animals later allocated to carvedilol treatment during cardioplegic arrest demonstrated by SVR_i (Table 2). Both CPB and cardioplegic arrest have a major cardiovascular impact that would most probably conceal this randomization bias. Nevertheless, caution should be taken with regard to interpretation of the afterload difference demonstrated after CPB in the present study.

Reperfusion is associated with an increase in formation of ROS due to sudden resupply of oxygen and decay in the antioxidant system. Also, CPB and cardioplegic arrest promote neutrophil tissue migration increasing ROS.[9] The imbalance between ROS and antioxidative defense initiates several reactions, ultimately leading to cell death and is thus a major mechanism behind reperfusion injury. Carvedilol and its

Table 4. Variables After 100 Minutes of Cardioplegic Arrest and Weaning From Cardiopulmonary Bypass

				RM-ANOVA Statistics		
Variable	1 Hour	2 Hours	3 Hours	p_w	p_g	p_i
LV-ESP (mmHg)						
Carvedilol	96 ± 6	86 ± 2	79 ± 4	<0.001	0.023	0.96
Control	84 ± 4	73 ± 3	68 ± 4			
LV-ESV_i (mL/m^2)						
Carvedilol	28 ± 4	23 ± 2	20 ± 3	0.18	0.98	0.28
Control	24 ± 4	25 ± 5	22 ± 3			
LV-EDP (mmHg)						
Carvedilol	7.9 ± 0.7	7.6 ± 0.9	8.2 ± 0.7	0.65	0.47	0.76
Control	7.4 ± 0.8	7.1 ± 0.7	7.2 ± 0.8			
LV-EDV_i (mL/m^2)						
Carvedilol	67 ± 4	56 ± 4	50 ± 4	<0.001	0.78	0.69
Control	65 ± 5	59 ± 6	53 ± 5			
SV_i (mL/m^2)						
Carvedilol	38 ± 1	34 ± 2	30 ± 2	<0.001	0.52	0.36
Control	42 ± 2	34 ± 2	31 ± 2			
LV-EF (%)						
Carvedilol	59 ± 4	61 ± 2	61 ± 3	0.49	0.59	0.24
Control	66 ± 4	61 ± 4	60 ± 3			
LV-SW_i ($mmHg \cdot mL/m^2$)						
Carvedilol	3,241 ± 179	2,682 ± 158	2,121 ± 193	<0.001	0.63	0.51
Control	3,299 ± 255	2,399 ± 247	1,999 ± 199			
RV-EDV_i (mL/m^2)						
Carvedilol	138 ± 7	125 ± 9	122 ± 11	<0.001	0.37	0.26
Control	148 ± 7	141 ± 9	127 ± 8			
RV-EF (%)						
Carvedilol	30 ± 1	27 ± 1	27 ± 1	0.10	0.64	0.98
Control	29 ± 1	26 ± 2	27 ± 1			
LV-blood flow						
Carvedilol*	1.22 ± 0.06	0.99 ± 0.07	0.96 ± 0.08	0.005	0.65	0.43
Control	1.10 ± 0.06	0.92 ± 0.12	1.01 ± 0.11			
RV-blood flow						
Carvedilol*	1.22 ± 0.08	1.13 ± 0.13	1.09 ± 0.11	0.21	0.66	0.51
Control	1.29 ± 0.12	1.09 ± 0.12	1.24 ± 0.12			
STE-CircS (%)						
Carvedilol	−14.7 ± 0.8	−15.7 ± 1.5	−13.7 ± 1.1	0.03	0.38	0.17
Control	−17.8 ± 1.2	−15.4 ± 0.8	−13.8 ± 0.9			
STE-LongS (%)						
Carvedilol	−10.9 ± 1.1	−9.2 ± 0.7	−7.7 ± 1.0	0.002	0.98	0.78
Control	−10.6 ± 1.1	−9.3 ± 0.6	−8.1 ± 0.8			
STE-RadS (%)						
Carvedilol	24.3 ± 2.3	23.0 ± 2.5	20.7 ± 1.4	0.013	0.14	0.37
Control	29.6 ± 2.8	27.2 ± 2.2	20.4 ± 1.7			
STE-CircSr (s^{-1})						
Carvedilol	−1.17 ± 0.07	−1.46 ± 0.13	−1.37 ± 0.15	0.78	0.31	0.041
Control	−1.41 ± 0.11	−1.24 ± 0.08	−1.26 ± 0.06			
STE-LongSr (s^{-1})						
Carvedilol	−1.07 ± 0.06	−1.05 ± 0.08	−1.13 ± 0.10	0.76	0.73	0.58
Control	−1.10 ± 0.08	−1.03 ± 0.04	−1.03 ± 0.06			
STE-RadSr (s^{-1})						
Carvedilol	2.31 ± 0.17	2.21 ± 0.24	2.27 ± 0.23	0.83	0.67	0.97
Control	2.19 ± 0.13	2.15 ± 0.12	2.16 ± 0.19			
PAP (mmHg)						
Carvedilol	26 ± 2	25 ± 1	22 ± 2	0.008	0.49	0.85
Control	27 ± 1	25 ± 1	24 ± 1			
CVP (mmHg)						
Carvedilol	6.5 ± 0.8	5.6 ± 0.3	6.0 ± 0.4	0.089	0.80	0.78
Control	6.8 ± 1.0	5.5 ± 0.6	6.6 ± 1.3			
LV-PFR/EDV (s^{-1})						
Carvedilol	6.0 ± 0.5	6.8 ± 0.9	7.3 ± 0.9	0.056	0.89	0.30
Control	6.5 ± 0.9	6.9 ± 1.0	7.2 ± 0.9			

Table 4 (*continued*)

Variable	1 Hour	2 Hours	3 Hours	RM-ANOVA Statistics p_w	p_g	p_i
SVR_i ($dyn \cdot s/cm^5 \cdot m^2$)						
Carvedilol	1,526 ± 125	1,475 ± 98	1,280 ± 104	<0.001	0.092	0.73
Control	1,304 ± 110	1,184 ± 120	1,022 ± 95			

NOTE. Variables after 100 min of cardioplegic arrest and weaning from cardiopulmonary bypass. Values are mean ± SEM, n = 10 in both groups.

Abbreviations: Circ, circumferential direction; CVP, mean central venous pressure; EDP, end-diastolic pressure; EDV, end-diastolic volume; EF, ejection fraction; ESP, end-systolic pressure; ESV, end-systolic volume; i, value indexed for body surface area; Long, longitudinal direction; LV, left ventricle; PAP, mean pulmonary artery pressure; PFR, peak filling rate; p_g, p value between groups from 2-way RM-ANOVA; p_i, p value for interaction from 2-way RM-ANOVA; p_w, p value within subjects from 2-way RM-ANOVA; Rad, radial direction; RM-ANOVA, analyses of variance for repeated measurement; RV, right ventricle; S, peak systolic strain; Sr, peak systolic strain rate; STE, left ventricular Speckle tracking echocardiography; SV, stroke volume; SVR, systemic vascular resistance; SW, stroke work.

*n = 9 in Carvedilol group.

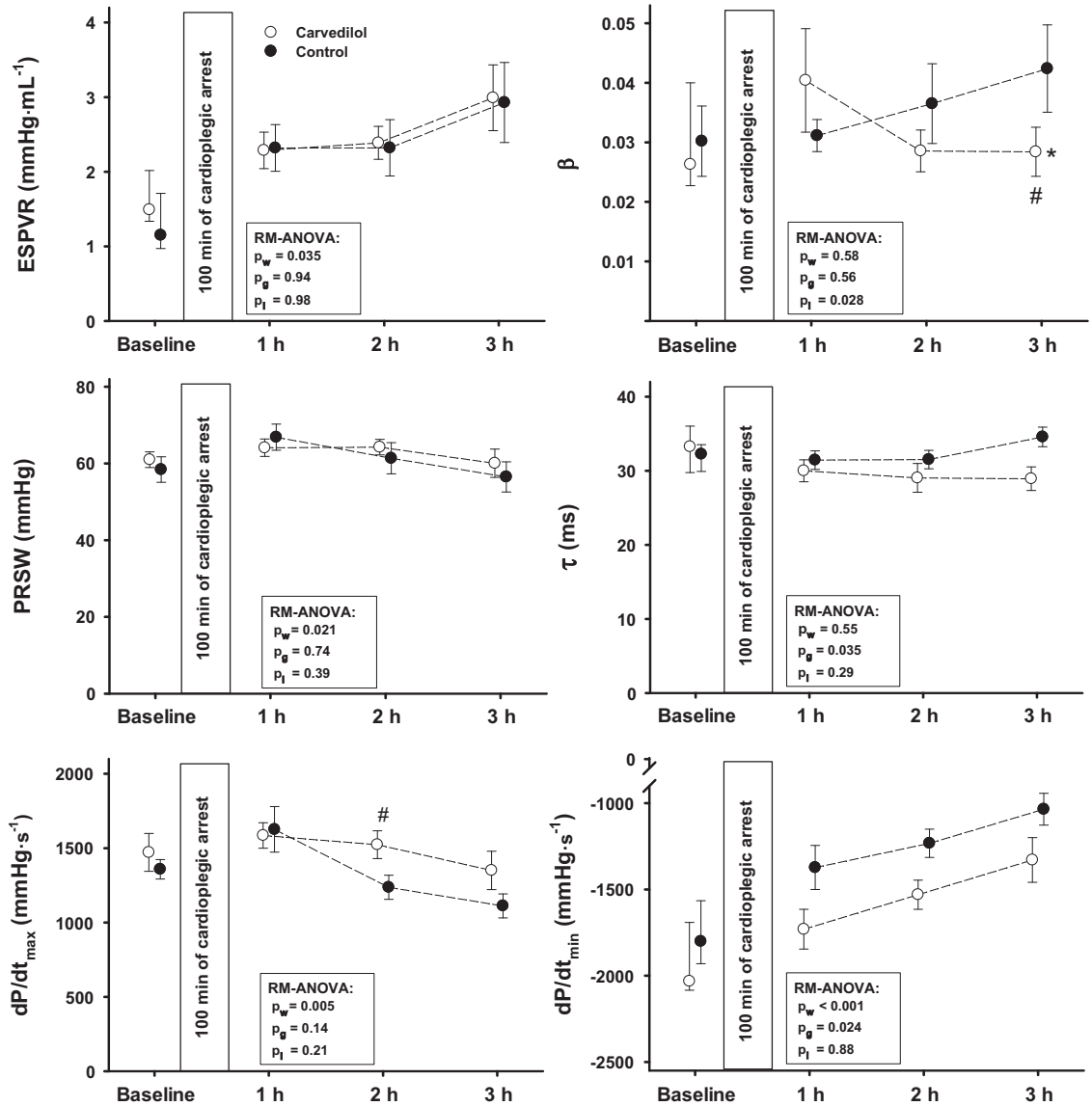

Fig 3. Slope of end-systolic pressure-volume relationship (ESPVR), slope of the logarithmic end-diastolic pressure-volume relationship (β), slope of preload recruitable stroke work (PRSW), isovolumic relaxation time constant (τ), and peak-positive and peak-negative of first derivative of left ventricular pressure (dP/dt_{max} and dP/dt_{min}) at baseline, 1 hour, 2 hours, and 3 hours after aortic declamping. Values are mean ± SEM or median with quartiles. p_w, p_g, and p_i = significance level for within, between groups, and interaction, respectively, by analyses of variance for repeated measurement. # Difference between groups at denoted time. * Difference within same group between 1 and 3 hours.

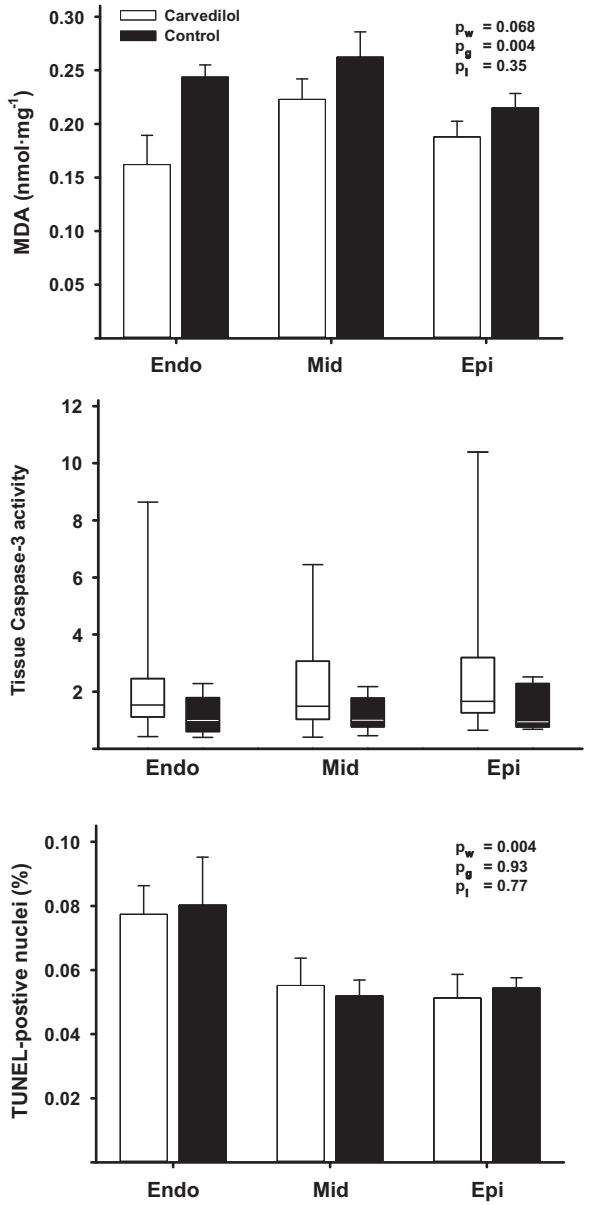

Fig 4. Malondialdehyde (MDA), caspase-3 activity, and TUNEL-positive nuclei from left ventricular tissue samples in subendocardial (ENDO), midmyocaridal (MID), and subepicardial (EPI) layers. p_w, p_g, and p_i = significance level for within subjects, between groups, and interaction, respectively, by RM-ANOVA. Values are mean + SEM and box-and-whisker plot for caspase-3.

hydroxylated metabolite are known to exhibit several antioxidant effects.[10] The authors' study showed less MDA in the intervention group, indicating reduced lipid peroxidation (Fig 4). However, neither apoptosis evaluated by caspase-3 activity and TUNEL-staining nor ischemic myocardial injury evaluated by serum troponin release differed between groups. In a study using human cardiomyocytes from the right atrium in a perfusion chamber simulating cardioplegic arrest, significantly better preservation with regards to apoptotic activation was shown when cells were treated with 10 μM of carvedilol.[13] Likewise, a systemic injection of 1.0 mg/kg of carvedilol just prior to CPB followed by 1 hour of cardioplegic arrest caused less apoptotic activation, corresponding to lower MDA levels in a canine model.[12] Furthermore, several studies have shown marked reduction in infarct size in coronary artery occlusion/reperfusion models.[2-4]

The lack of clear evidence for myocardial salvage in the present study may be dose dependent. Based on plasma volume of 52 mL/kg in the young pig,[33] peak total plasma concentrations of carvedilol (free and protein bound) in the previously-mentioned studies range, between 10 μM and 45 μM. This was significantly more than used in the present study, in which calculated carvedilol concentrations were 5.6 μM and 3.1 μM for high- and low-dose cardioplegia, respectively (Table 1). However, a direct comparison of systemic bolus injections and freshly mixed intracoronary delivery probably is not valid since several factors like distribution volume, blood temperature and time to equilibrium of plasma protein-bound fraction will be different. In addition, the repeated perfusions of intracoronary cardioplegia allow a targeted intervention to the myocardium during the ischemic and reperfusion periods. In pilot experiments, double and quadruple doses of carvedilol in the cardioplegic solution were associated with unacceptable prolonged asystolic washout periods and problems with weaning from CPB. Thus, the final concentrations of carvedilol used in the present study were titrated to be clinically relevant for the intracoronary delivery approach.

Limitations

Translating the results from this animal study to a clinical situation in humans should be done with care. The healthy pig hearts have not been subjected to a direct surgical intervention. Many patients undergoing cardiac surgery have a previous history of ischemic heart disease, myocardial infarction, hypertrophy and dysfunction that have not been modeled here. However, the mechanisms explaining reduced antioxidative stress combined with improved diastolic function in the present study were not species specific. There may very well be similar advantages utilizing carvedilol-enriched cardioplegia in a clinical setting, but caution must be taken in choice of dose as cardiodepression before total washout of cardioplegic remnants may prolong weaning times. It should also be emphasized that the statistical power in the present study was low, and negative findings must be interpreted with caution.

CONCLUSIONS

In the authors' pig model, carvedilol used as additive to the cardioplegic solution reduced oxidative stress in the myocardium. Carvedilol preserves diastolic function during the first 3 hours after CPB and cardioplegic arrest. However, the authors found no evidence of definitive protection from ischemic and lethal reperfusion injury.

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APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1053/j.jvca.2016.03.152.

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