

Myocardial Protective Effect of Warm Blood, Tepid Blood, and Cold Crystalloid Cardioplegia in Coronary Artery Bypass Grafting Surgery

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- Aim** To compare the myocardial effects of cardioplegia by warm blood, tepid blood, and cold crystalloid during coronary artery bypass grafting (CABG).
- Methods** Patients undergoing CABG surgery at Kaunas University Hospital between 2000 and 2004 were randomized into three groups (n=156), receiving a different method of cardioplegia. Intermittent antegrade warm blood cardioplegia was used in 51 patients, tepid blood cardioplegia in 50 patients, and cold crystalloid cardioplegia in 55 patients. Mitochondrial function, myocardial ultrastructure, troponin T, and hemodynamic and clinical data were analyzed after surgery.
- Results** All cardioplegic methods similarly affected structural and functional properties of mitochondria and coupling of oxidative phosphorylation, and all lowered the capacity of mitochondria to synthesize ATP. Ultrastructure of myocytes showed slight to moderate injury in the cold crystalloid cardioplegia group. The concentration of troponin T was significantly lower in the warm blood cardioplegia group than in the tepid blood cardioplegia and cold crystalloid cardioplegia groups at 12 hours (0.8 ± 0.1 ng/mL, 1.9 ± 0.2 ng/mL, and 2.8 ± 0.3 ng/mL, respectively; $P < 0.001$) and 24 hours after surgery (1.0 ± 0.1 ng/mL, 2.2 ± 0.3 ng/mL, and 2.5 ± 0.3 ng/mL, respectively; $P < 0.001$). Echocardiographic examination after surgery revealed that the changes in the left ventricle diastolic function were similar in all groups, and that systolic function did not change. The warm blood cardioplegia group showed shorter duration of intubation and hospitalization. There were no differences in the need of catecholamine administration, incidence of complications, and duration of stay in the intensive care unit.
- Conclusions** Intermittent antegrade warm blood cardioplegia provides better myocardial protection during CABG surgery, as assessed by the lower release of troponin T, lower fluid balance, shorter duration of tracheal intubation and hospital stay.

A tremendous body of literature has documented the importance of physiologic approach of current cardioprotective methods and debate over the optimal technique. Many cardioplegic techniques employ cold crystalloid solutions or blood to initiate and maintain intraoperative cardiac arrest (1). Cold crystalloid cardioplegia, in addition to facilitating adequate visualization of the operation field, provides the presumed advantage

of inhibiting myocardial metabolic and enzymatic activity when perfusion of the heart is suboptimal (1). Further studies suggested that the delay in metabolic and functional cardiac recovery was secondary to the hypothermic inhibition of myocardial enzymes and warm induction of cardioplegic arrest improved myocardial metabolic and functional recovery after coronary artery bypass grafting (CABG) (2). After the report by Lichtenstein et

al (3), continuous warm blood cardioplegia has been routinely used in a growing number of cardiac surgery centers. However, as the bleeding into the operating field led to the interruption of the infusion of warm blood, Calafiore et al (4) proposed intermittent antegrade warm blood cardioplegia as a safe and reliable technique of myocardial protection.

We decided to compare three cardioplegic techniques – warm blood, tepid blood, and cold crystalloids – used in CABG for their protective effect on myocardial tissue.

Patients and Methods

Patients

The study included 195 patients undergoing elective CABG surgery at Kaunas University Hospital between 2000 and 2004. All patients eligible and scheduled for CABG surgery were included in the study. Because troponin T was shown to be a more sensitive marker of myocardial cell damage and to be affected by muscle lesions or renal insufficiency (5,6), the patients with renal insufficiency and/or cardioversion before the surgery were not included in this study. Computer-generated random numbers were used to randomize the patients into three groups. There were 64 patients in the warm blood cardioplegia group, 62 patients in the tepid blood cardioplegia group, and 69 patients in the cold crystalloid cardioplegia group. We perioperatively excluded 5 (7.8%) and 8 (12.5%) patients from warm blood cardioplegia group, 4 (6.5%) and 8 (12.9%) patients from tepid blood cardioplegia group, and 4 (5.8%) and 10 (14.5%) patients from cold crystalloid cardioplegia group for renal insufficiency and cardioversion, respectively. Intermittent antegrade warm blood cardioplegia was used in 51 patients, intermittent antegrade tepid blood cardioplegia in 50 patients, and intermittent antegrade cold crystalloid cardioplegia was applied in 55 patients. There were no significant differences in the clinical characteristics between the groups before the surgery (Table 1). The study was approved by the Ethics Committee and all patients provided their informed consent before inclusion into the study.

Anesthesia, Surgical Procedures, and Perfusion

Anesthesia was induced with midazolam (0.05 mg/kg), thiopental (1-3 mg/kg) and

Table 1. Preoperative and operative clinical characteristics of the patients undergoing coronary artery bypass grafting

Clinical characteristics*	Method of cardioplegia		
	warm blood (n=51)	tepid blood (n=50)	cold crystalloid (n=55)
Men/women	40/11	41/9	43/12
Age (mean±SD, years)	63±9	64±11	62±10
LVEF (mean±SD, %)	43.8±7.1	42.9±7.0	42.8±7.7
NYHA class (No. of patients):			
III	43	41	45
IV	8	9	10
Comorbid conditions (No. of patients):			
hypertension	22	23	21
diabetes mellitus	4	3	2
arrhythmias	2	3	4
other	4	4	9
Aortic cross-clamp time (mean±SD, min)	48±15	51±13	49±8
CPB time (mean±SD, min)	93±19	100±21	95±17
Duration of operation (mean±SD, min)	216±35	213±42	214±42
No. of distal anastomoses (mean±SD)	3.4±0.7	3.6±0.7	3.7±0.7

*Abbreviations: LVEF – left ventricle ejection fraction, NYHA – New York Heart Association, CPB – cardiopulmonary bypass. There were no statistically significant differences among the groups (χ^2 -test for categorical and t-test for continual variables).

fentanyl (1-2 μ g/kg), and muscle relaxation with pipekuronium bromide (0.08-0.1 mg/kg). Anesthesia was maintained with fentanyl at the dosage of 3 μ g/kg/h (~10-20 μ g/kg in total), midazolam (1-4 mg/h), pipekuronium (0.01 mg/kg/h IV), and isoflurane gas at 0.5 to up to 0.75 minimal alveolar concentration, depending on the hemodynamic data.

Cardiopulmonary bypass was established by cannulation of the ascending aorta and a single double-staged right atrial cannulation. Hollow fiber membrane oxygenator (Compactflo D703; Dideco, Miranda, Italy) was used for blood oxygenation. The cardiopulmonary bypass circuit was primed with 2 L of lactated Ringer's solution. Systemic heparin (300 U/kg) was administered to achieve an activated clotting time of >450 s.

The same surgical technique was used in all three groups. All operations were performed via median sternotomy. The left internal thoracic artery and venous grafts were used as graft material. Distal anastomoses were constructed during the total aortic cross-clamp. Proximal anastomoses were performed by partial side-bite clamping of the ascending aorta.

Cardioplegic Methods

Cardioplegia was delivered into the aortic root immediately after the aortic cross-clamping in all patients.

In the warm blood cardioplegia group, cardiac arrest was achieved by intermittent infusion of normothermic blood. The blood was collected from an oxygenator and infused by a roller pump. The tubing was connected to a syringe pump containing 15% potassium chloride solution. The initiation of cardiac arrest was achieved by a roller pump flow rate of 300 mL/min and a bolus injection of 3 mL of KCl followed by a continuous infusion of 150 mL/h from the syringe pump. The initial application time of this cardioplegic solution was 2 minutes. The time period between two infusions, which define an ischemic arrest period, never exceeded 15 minutes. The roller pump flow rate was 200 mL/min at each of the following cardioplegic arrests, whereas the continuous infusion from the syringe pump was reduced to 120 mL/h, 90 mL/h, and 60 mL/h in the second, third, and following cardioplegic arrests. During cardiopulmonary bypass, the blood temperature was allowed to drop to 36°C on average.

In the tepid blood cardioplegia group, cardioplegic tepid solution (28-30°C) was intermittently infused into the aortic root every 15 minutes. This solution contained oxygenated blood from the oxygenator and crystalloid solution in 4:1 ratio, and was administered over 2 minutes.

In the cold crystalloid cardioplegia group, cardiac arrest was achieved by intermittent infusion of 1 L of cold (4°C) crystalloid solution containing K⁺ (24 mmol), Na⁺ (110 mmol), Ca²⁺ (1.8 mmol), and Cl⁻ (160 mmol) into the aortic root. Additional 500 mL of the solution were administered every 30 minutes. Body temperature was allowed to drop to a minimum of 30°C. Re-warming was initiated during the completion of the last distal anastomosis.

Assessment of Mitochondrial Function

The human right atrial appendage tissue for measurements of mitochondrial respiration (20-mg samples) was obtained from the patients before cardioplegia and after the aorta clamp removal, within 10-15 minutes after reperfusion. The saponin+collagenase-permeabilized fibers were prepared and investigated as described by Toleikis et al (7) and Liobikas et al (8). Respiration rates, with 15 mmol/L succinate as a respiratory substrate and 5 µmol/L rotenone, were expressed as ng-atom O/min/mg of dry weight of fibers (9,10).

Electron Microscopy Studies

To determine the ultrastructural changes in the myocardium during cardioplegia in each patient, two fine needle biopsies from the left ventricle were performed, one just before the aorta cross-clamping and the other after the removal the clamp (10-15 minutes after reperfusion). The biopsy samples were fixed in 2.5% glutaraldehyde solution in 0.1 molar cacodylate buffer (pH 7.3), cooled at 4°C, and prepared according to routine methods of electron microscopy (11). Ultra-thin sections stained with uranyl acetate and lead citrate were examined by using electron microscope Tesla 500 BS (Tesla, Prague, Czech Republic). A semiquantitative method of the standard grading was used for ultrastructural assessment of the myocardial samples (12,13). Eight grades of ischemic injury were defined with respect to granulae, cristae and matrix of the mitochondria, swelling and integrity of the nuclei, myofibrils, organelles, membranes, and capillaries, as follows (14): grade 1 – normal ultrastructural appearance; grade 2 – normal to slight injury, reversible; grade 3 – slight ischemic injury, reversible; grade 4 – slight to moderate injury, reversible; grade 5 – moderate ischemic injury, reversible; grade 6 – moderate to severe injury, reversible; grade 7 – severe ischemic injury, reversible; grade 8 – irreversible ischemic injury. Grading was performed in a blinded fashion.

Troponin T Measurements

Troponin T was measured in blood samples taken before surgery and at 12 and 24 hours after surgery for credible maximum values according to the recommendations of Biochemical Laboratory, University of Kaunas. Troponin T in serum was quantitatively determined by immunoelectrochemiluminescence assay (Boehringer Mannheim Elecsys 1010, Mannheim, Germany). It is generally accepted that troponin T concentration >0.1 ng/mL shows slight myocardial injury, troponin T concentration >1.0 ng/mL shows moderate myocardial injury, and troponin T concentration >3.0 ng/mL shows massive myocardial injury (15).

Evaluation of Hemodynamics

The left ventricular systolic and diastolic function parameters were echocardiographically assessed before and up to 10 days after surgery. The left ventricle (LV) end-diastolic diameter and ejection fraction were measured according to the

guidelines of the American Association of Echocardiography (16). Segmental LV motion was evaluated by a semi-quantitative method according to the model of 16 segments of LV, in a 5-score system, by calculating the wall motion score (16). LV diastolic filling was evaluated from the 4-chamber view with the pulse Doppler sample volume at the leaflets of the opened mitral valve. The measurements included peak velocity of the early (E) and late (A) transmitral flow, E velocity deceleration time (DT_E); the E/A ratio was calculated (17). All measurements were performed in 3-5 cardiac cycles and averaged.

Clinical Data

Clinical data evaluation was based on the administration of catecholamines in the post-operative period, duration of intubation, fluid balance in the operating room, the incidence of complications, and duration of stay in intensive care unit (ICU) and hospital.

Colloids (gelatin or hydroxyethyl starch) were infused if central venous pressure was < 12 mm Hg and if one of the following criteria was met: mean blood pressure < 65 mm Hg, heart rate > 110 beats/min, and urine output < 0.5 mL/kg/h. If these values were not reached after volume loading, catecholamines were administered. If hemodynamic parameters did not reach the defined values after epinephrine was administered at increasing dosage (low dose – 0.01-0.03 $\mu\text{g}/\text{kg}/\text{min}$, moderate dose – 0.04-0.09 $\mu\text{g}/\text{kg}/\text{min}$,

and high dose – > 0.1 $\mu\text{g}/\text{kg}/\text{min}$), dopamine or dobutamine were used.

Extubation was performed as soon as the patients were able to maintain adequate gas exchange, stable hemodynamic parameters, and if they were adequately rewarmed and not bleeding.

Neurological complications were evaluated by a neurologist, whereas computerized tomography scan was performed if necessary.

All patients had to meet the same criteria for discharge from the ICU and hospital.

Statistical Analysis

Continuous variables were expressed as mean with either standard deviation (\pm SD) or 95% confidence intervals (95% CI). Student's *t*-test and paired samples *t*-test were used accordingly for the comparison of the means for two independent and two related samples. Analysis of variance (ANOVA) was used to compare means for more than two groups. To compare discrete variables, we used Pearson's χ^2 test or Fisher's exact test. *P* value of < 0.05 was considered statistically significant. Statistical analysis was performed by using GraphPad Prism statistical package (ver. 4.00 for Windows, GraphPad Software, 2002, San Diego, CA, USA).

Results

Mitochondrial Respiration

We present typical oxygraphic trace of saponin+collagenase-skinned fibers from atrium (Fig. 1). For evaluation of mitochondrial oxygen

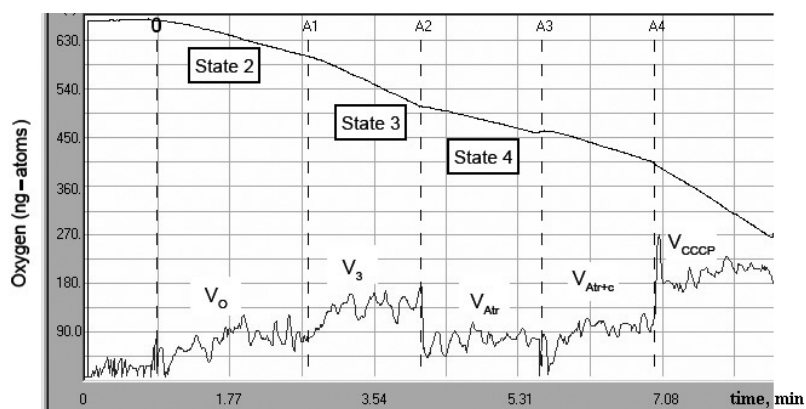


Figure 1. The scheme of oxygraphic measurements of the saponin+collagenase-treated heart atrium fibers. The upper trace indicates the oxygen concentration in the oxygraph chamber, the lower trace represents the first derivative of these signals indicating the respiratory rate. Substrate: 15 mmol/L succinate (+5.3 $\mu\text{mol}/\text{L}$ rotenone). Order and final concentration of additions: 0 (V_0) – fibers (5 mg); A1 (V_3) – 1 mmol/L adenosine triphosphate (ADP); A2 (V_{Atr}) – 0.12 mmol/L atractyloside; A3 ($V_{\text{Atr+c}}$) – 32 $\mu\text{mol}/\text{L}$ cytochrome c; A4 (V_{CCCP}) – 1.2 $\mu\text{mol}/\text{L}$ carbonyl cyanide m-chlorophenylhydrazone (CCCP).

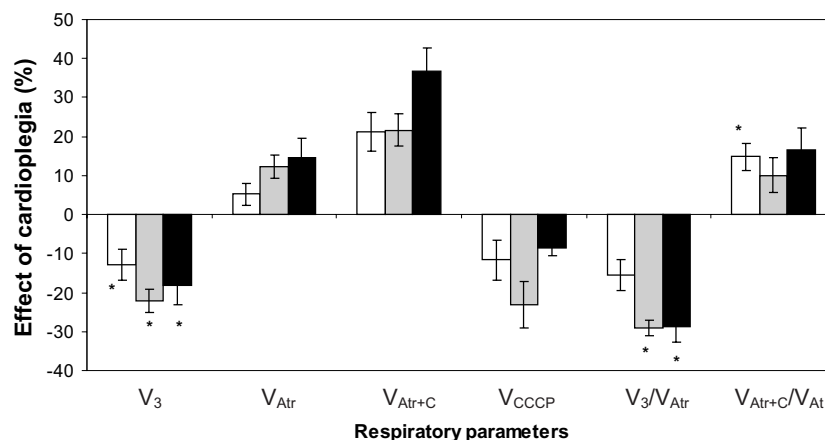


Figure 2. Effects of cardioplegia on changes in mitochondrial respiratory parameters in human atrial mitochondria. Open column – warm blood cardioplegia group, gray column – tepid blood cardioplegia group closed column – cold crystalloid cardioplegia group. Supplements were added in the following order: 15 mmol/L succinate + 5.3 μ mol/L rotenone; 1 mmol/L ADP (V_3); 105 μ mol/L atractyloside (V_{Atr}); 15.4 μ mol/L cytochrome c (V_{Atr+C}); 2.1 μ mol/L carbonyl cyanide m-chlorophenylhydrazone (V_{CCCP}); respiratory control index (V_3/V_{Atr}); effect of cytochrome c (V_{Atr+C}/V_{Atr} ; times). Respiratory rate in ng-atom O/min/mg of dry weight of fibers. *t*-test for dependent samples was used. Asterisk indicates $P < 0.05$ compared with baseline.

consumption in State 2, fibers were placed in incubation medium supplemented with saturated concentration of respiratory substrate succinate. Maximum respiration rate (State 3) measured in the presence of ADP and inorganic phosphate (Pi) reflect the capacity of mitochondria to synthesize ATP (18). State 4 respiration rate (V_{Atr}) was measured after the addition of atractyloside, which inhibited ADP/ATP-translocator and blocked the access of ADP to mitochondria, resulting in a decrease in the State 3 respiration rate. The intactness of the outer mitochondrial membrane was checked by addition of cytochrome c during State 4 respiration. Finally added carbonyl cyanide m-chlorophenylhydrazone (CCCP) acts as uncoupler and allows respiration to continue without ATP synthesis (18). V_{CCCP} gives the information about maximum activity of mitochondrial respiratory chain (18).

All cardioplegic methods – warm blood, tepid blood, and cold crystalloid – lowered the capacity of mitochondria to synthesize ATP (Fig. 2). This occurred mainly due to the decrease by 13-22% in the maximum respiration rate (V_3). We found an increase by 5-15% in the non-phosphorylating (i.e., not related with ATP synthesis) State 4 respiration rate (V_{Atr}), which was more pronounced in the presence of cytochrome c (V_{Atr+C}) (Table 2, Fig. 2). The changes of these pa-

rameters in the opposite direction caused the decrease in respiratory control index (V_3/V_{Atr}), calculated as the ratio between the State 3 to the State 4 respiration rates. These data reflected the injury and the increase in the ion permeability of inner mitochondrial membrane, the partial loss of coupling between respiration and oxidative phosphorylation, and the decrease in the ATP production rate in the cardioplegia-affected atrial fibers.

The intactness of outer mitochondrial membrane was evaluated by the subsequent addition of exogenous cytochrome c, the component of mitochondrial respiratory chain, in the State 4. Since the intact membrane is not permeable to cytochrome c, no stimulation of respiration should be observed in the case of intact membranes (18). When cytochrome c was added into the incubation medium after the measurement of State 4 respiration rate (V_{Atr}), we observed the stimulation of respiration of fibers even before cardioplegia (V_{Atr+C}/V_{Atr}) by 23-37% in all groups (Fig. 2). This indicated a slight injury of mitochondrial outer membrane, which further increased after cardioplegia (Table 2). This finding, together with the disturbances of the State 3 and uncoupled respiration (V_{CCCP}), which reflects the maximum activity of mitochondrial respiratory chain, indicated cardioplegia-induced damage to the outer mitochondrial membrane and a related release of cytochrome c

Table 2. Influence of cardioplegia on mitochondrial respiratory parameters in the atrial fibers from our patients undergoing coronary artery bypass grafting

Method of cardioplegia†	Mitochondrial respiratory parameters (95% CI)*					
	V ₃	V _{ATr}	V _{ATr+C}	V _{COCP}	V ₃ /V _{ATr}	V _{ATr+C} /V _{ATr}
Warm blood:						
before	52.9 (45.3-60.7)	17.5 (14.9-20.2)	21.3 (19.2-23.6)	49.5 (39.6-59.8)	3.09 (2.71-3.45)	1.23 (1.15-1.32)
after	45.8 (37.3-54.4)	18.3 (14.7-21.7)	25.8 (19.8-31.5)	43.7 (32.1-55.1)	2.55 (2.19-3.03)	1.41 (1.25-1.58)
P	0.038	0.623	0.098	0.055	0.110	0.013
Tepid blood:						
before	55.3 (44.2-67.0)	18.8 (14.0-25.1)	25.3 (18.4-30.9)	56.4 (42.0-69.0)	3.14 (2.14-3.85)	1.37 (1.12-1.46)
after	43.1 (33.1-54.4)	20.5 (16.5-24.7)	30.3 (16.8-42.7)	42.9 (32.0-67.6)	2.16 (1.84-2.47)	1.50 (1.03-1.81)
P	0.001	0.475	0.284	0.069	0.015	0.403
Cold crystalloid:						
before	70.0 (61.0-75.6)	22.3 (17.7-25.7)	29.7 (23.3-34.7)	78.5 (66.7-85.8)	3.33 (2.76-3.84)	1.36 (1.21-1.50)
after	57.1 (47.7-67.9)	25.5 (19.9-31.7)	40.3 (28.2-51.9)	72.4 (55.7-89.6)	2.30 (2.04-2.59)	1.58 (1.23-1.89)
P	0.012	0.065	0.074	0.459	0.003	0.189

*Respiratory rate (V) expressed in ng·atom O/min/mg of dry weight of fibers. Supplements were added in the following order: 15 mmol/L succinate + 5.3 μmol/L rotenone; V₃ - 1 mmol/L ADP; V_{ATr} - 105 μmol/L atractyloside; V_{ATr+C} - 15.4 μmol/L cytochrome c; V_{COCP} - 2.1 μmol/L carbonyl cyanide m-chlorophenylhydrazone; V₃/V_{ATr} - respiratory control index; V_{ATr+C}/V_{ATr} - effect of cytochrome c (time).

†t-test for dependent samples; before - before cardioplegia; after - after the aorta cross clamp release.

from mitochondria (this is our conclusion based on our results presented above). This, at least in cold crystalloid cardioplegia group, largely determined the decrease in the maximum ADP-stimulated respiration rate of mitochondria (V₃) (Fig. 2).

Electron Microscopy

Ultrastructural investigation of biopsy samples from the left ventricle revealed that heart arrest in the warm blood and tepid blood cardioplegia groups did not cause any significant cell damage. Median grade of myocardial injury in the warm blood, tepid blood, and cold crystalloid cardioplegia groups before the cardioplegia was 2 (range, 1-4), 2 (range, 1-4), and 2 (range, 1-3), respectively, and after the cardioplegia, it was 2 (range, 1-5), 3 (range, 1-6) and 4 (range, 1-7), respectively. The mean grade of ischemic injury to the left ventricular myocyte structure in the warm blood, tepid blood, and cold crystalloid cardioplegia groups was 2.0±1.4, 2.1±1.5, and 2.1±0.9 before cardioplegia (control values) and 2.3±1.4, 2.7±0.5, and 4.1±1.5 after aorta cross-clamp release, respectively. The only significant increase was found in the cold crystalloid group (P=0.005, t-test for dependent samples).

Troponin T

The concentration of troponin T at 12 and 24 h after surgery was 2.8±0.3 ng/mL and 2.5±0.3 ng/mL in the cold crystalloid cardioplegia group, 1.9±0.2 ng/mL and 2.2±0.3 ng/mL in the tepid blood cardioplegia group, and 0.8±0.1 ng/mL and 1.0±0.1 ng/mL in the warm blood cardioplegia group, respectively. Troponin T blood concentration was significantly lower in

the warm blood cardioplegia group than in the tepid blood and cold crystalloid cardioplegia groups (P<0.001 for both) at 12 and 24 hours postoperatively. Also, troponin T blood concentration was significantly lower in the tepid blood cardioplegia group than in the cold crystalloid cardioplegia group at 12 hours postoperatively (P<0.001; Fig. 3).

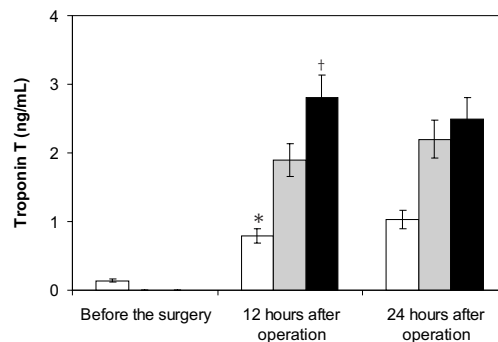


Figure 3. Effects of cardioplegia on the dynamics of troponin T concentration in blood serum. Open bars - warm blood cardioplegia group, gray bars - tepid blood cardioplegia group, closed bars - cold crystalloid cardioplegia group. *P<0.005, warm blood cardioplegia group compared with tepid blood and cold crystalloid cardioplegia groups; †P<0.005, tepid blood cardioplegia group compared with cold crystalloid cardioplegia group (analysis of variance).

Hemodynamic Parameters

Echocardiographic examination revealed that the postoperative LV diastolic function and systolic function was similar in all groups. Early transmitral blood flow velocity significantly changed postoperatively as compared to before

the surgery in all three groups, as well as early to late transmitral blood flow velocity ratio (Table 3).

Clinical Data

The analysis of clinical data revealed that 35 out of 51 patients in the warm blood cardioplegia group, 35 out of 50 in the tepid blood cardioplegia group, and 36 out of 55 in the cold crystalloid cardioplegia group did not require catecholamines in the early postoperative period. Low doses of catecholamines were administered to 13 patients in warm blood cardioplegia group, 11 in the tepid blood cardioplegia group, and 5 in the cold crystalloid cardioplegia group. Moderate and high doses of catecholamines were mostly administered to the patients in the cold crystalloid cardioplegia group (13 vs 4 in the warm blood cardioplegia and 5 in the tepid blood cardioplegia group). There were no differences in the need for catecholamine administration between the tepid and warm blood cardioplegia groups ($P=0.741$).

Postoperative duration of intubation in the warm blood cardioplegia group was 37% shorter than in the tepid blood cardioplegia group (357 ± 155 min vs 567 ± 205 min, $P=0.014$). However, it was not different comparing the tepid blood cardioplegia group with the cold crystalloid cardioplegia group (567 ± 205 min vs 448 ± 180 min, $P=0.153$). Mean positive fluid balance during the surgery was $1,250 \pm 613$ mL in the warm blood cardioplegia patients, $1,989 \pm 733$ mL in the tepid blood cardioplegia patients, and $2,167 \pm 839$ mL in the cold crystalloid cardioplegia patients. It was revealed that the value of fluid balance was 42% higher in the cold crystalloid cardioplegia group than in the warm blood cardioplegia group ($P=0.005$). The fluid balance of warm blood cardioplegia patients during surgery was significantly lower than in tepid blood cardioplegia and cold crystalloid cardioplegia patients ($P=0.014$).

The outcome variables, especially the frequency of occurrence of neurological complications, the need for cardiac pacing at the end of the operation, reoperation because of bleeding during the early postoperative period, perioperative myocardial infarction, intraaortic balloon pump support, atrial fibrillation, and number of deaths did not differ among the three groups of patients (Table 4).

Table 4. Postoperative clinical parameters in patients who underwent different method of cardioplegia for coronary artery bypass grafting

Clinical parameters	Method of cardioplegia (No. of patients)*		
	warm blood (n=51)	tepid blood (n=50)	cold crystalloid (n=55)
Electrical pacing at the end of surgery	3	2	2
Reoperation for bleeding	1	2	3
Myocardial infarction	1	1	2
Intraaortic balloon counterpulsation	1	0	1
Atrial fibrillation	10	12	14
Deaths	1	1	1

*Differences among groups were not significant (χ^2 test).

The duration of ICU stay, which did not differ among the groups, was 38.9 ± 3.8 h in the warm blood, 45.1 ± 3.6 h in the tepid blood, and 44.2 ± 6.2 h in the cold crystalloid cardioplegia group. However, the duration of in-hospital stay was 26% shorter in the warm blood cardioplegia group than in the cold crystalloid cardioplegia group (13.5 ± 1.3 days and 18.3 ± 1.6 days, respectively; $P < 0.005$).

Discussion

Our results showed that warm blood cardioplegia provided better protection of myocardial tissue during CABG surgery than the two other methods, as shown by the release of cardiac-specific marker troponin T, shorter duration of intubation, and shorter duration of hospital stay of the

Table 3. Echocardiographic evaluation of the left ventricle function before and after coronary artery bypass grafting*

Index†	Method of cardioplegia (mean, 95% CI)								
	warm blood			tepid blood			cold crystalloid		
	before CABG	after CABG	P^{\ddagger}	before CABG	after CABG	P^{\ddagger}	before CABG	after CABG	P^{\ddagger}
E (cm/s)	63.8 (37.6-90.0)	78.6 (51.0-106.2)	0.003	53.9 (48.1-59.7)	79.5 (66.9-92.1)	0.005	53.1 (47.3-58.9)	72.4 (65.0-79.8)	0.025
A (cm/s)	80.0 (47.8-112.2)	68.4 (38.4-98.4)	NS	65.2 (60.2-70.2)	63.3 (53.9-72.7)	NS	73.6 (66.0-81.2)	64.8 (56.8-72.8)	NS
E/A	0.79 (0.51-1.07)	1.17 (0.37-1.97)	0.012	0.83 (0.73-0.93)	1.34 (1.14-1.54)	0.001	0.76 (0.64-0.88)	1.25 (0.99-1.51)	0.026
DT _E (s)	0.16 (0.14-0.18)	0.14 (0.12-0.16)	NS	0.19 (0.17-0.21)	0.19 (0.17-0.21)	NS	0.21 (0.19-0.23)	0.18 (0.16-0.20)	NS
LVEDD (mm)	47.7 (45.3-50.1)	48.6 (46.2-51.1)	NS	51.2 (48.8-53.6)	49.4 (47.1-51.7)	NS	48.1 (46.7-49.5)	47.7 (45.6-49.7)	NS
LVEF (%)	43.8 (41.8-45.8)	43.6 (41.6-45.6)	NS	42.9 (40.9-44.9)	43.3 (39.5-47.1)	NS	42.8 (40.6-45.0)	42.9 (40.5-45.3)	NS
WMS	1.5 (1.3-1.6)	1.4 (0.4-2.4)	NS	1.6 (0.5-0.7)	1.4 (1.3-26.0)	NS	1.4 (1.3-1.6)	1.4 (1.3-1.5)	NS

*Abbreviations: CABG - coronary artery bypass grafting; E - early transmitral blood flow velocity, A - late transmitral blood flow velocity, DT_E - duration of deceleration of E blood flow, LVEDD - end-diastolic diameter of the left ventricle, LVEF - left ventricular ejection fraction, WMS - wall motion score.

†t-test for dependent samples (before and after CABG), NS - not significant.

operated patients. All cardioplegic methods lowered the capacity of mitochondria to synthesize ATP and damaged the integrity of inner and outer mitochondrial membrane. The myocardial ultrastructural changes in the warm blood and tepid blood cardioplegia groups were not significant. The method of myocardial protection did not influence the hemodynamic data, the need for catecholamines administration during the early postoperative period, the incidence of complications, and the duration of ICU stay.

In our study, the myocardial cell damage was significantly reduced by the use of warm blood cardioplegia. Troponin T was significantly lower after normothermic blood cardioplegia than after either tepid blood or crystalloid cardioplegia at each time-frame point, which is in accordance with the observations of most other investigators (4,19). This difference may be due to the fact that the sampling was not done simultaneously – the troponin T concentration was measured 12 and 24 hours after operation, whereas mitochondrial function was measured 10-15 minutes after the restoration of blood circulation.

As reported by Birdi et al (20), normothermic systemic perfusion had no influence on gas exchange and ventilation variables in our study. Similarly to results obtained by Tonz et al (21), duration of intubation in our study was significantly shorter in normothermic group than in tepid blood cardioplegia group, which probably depended on a longer rewarming period and fluid overload in tepid blood and cold crystalloid cardioplegia patients. At the end of operation, despite increased diuresis, the cold crystalloid cardioplegia group had a significantly higher total fluid balance.

Length of ICU stay was the same in all groups. However, length of stay in the hospital was significantly shorter in the warm blood cardioplegia group comparing with cold crystalloid cardioplegia group. These very gross and non-specific markers of outcome are influenced by numerous external independent factors. It is possible but hardly probable that they are influenced by the cardioplegia or bypass temperature. For instance, because no stepdown unit is available in our hospital, patients were usually kept in the ICU until the second postoperative day.

The measurements of the respiration in saponin + collagenase-treated atrial appendage fi-

bers of the human heart by oxygraphic method allows the assessment of the functional activity and integrity of mitochondrial outer and inner membranes *in situ*, i.e., without the need for isolation of mitochondria. Mitochondrial membranes in fiber preparations are intact, in contrast to preparations of isolated mitochondria. It should be also noted that skinned-fiber technique is the only way to study mitochondria in the small human tissue biopsies (18).

All the methods of cardioplegia we used caused the inhibition of mitochondrial State 3 respiration rate and a non-significant increase in State 4 respiration rate. Furthermore, we determined the damage of the outer and inner mitochondrial membranes. The structural and functional disturbances of mitochondria led to the decrease in ATP production rate. The atrial appendage tissue was similar (statistically not different for all parameters studied) in all groups, although inner mitochondrial membrane integrity seemed to be less affected in the warm blood cardioplegia group. Hayashida et al (22,23) reported that cold cardioplegic solution decreased mitochondrial respiratory rate at the State 3 and caused functional heart recovery disorders at postoperative period, whereas warm and tepid cardioplegic solutions affected mitochondrial respiratory function less.

Our ultrastructural investigations of biopsies from heart ventricles revealed that heart arrest did not cause any significant cell damage. Myocardial protection achieved with warm blood cardioplegia was as good as that achieved with tepid blood cardioplegia. Myocardial structure was the same as before the cardiac arrest, or slightly reversibly changed. However, we observed significant changes showing slight to moderate injury within the myocardium ultrastructure after using cold crystalloid cardioplegia. These results are similar to those of Ferreira et al (11), who also found that blood cardioplegia offered better protection to the myocyte than crystalloid cardioplegia.

Echocardiographic examination of the left ventricle function revealed that the choice of method of cardioplegia did not influence hemodynamic parameters in our patients.

The proportion of patients requiring inotropic support or an intraaortic balloon pump was the same in all groups in our study. However, the proportion of our patients requiring inotropic support compared with those evaluated

by other investigators was higher (4,24). More liberal criteria for the use of inotropic drugs can explain the difference between the present study and previous reports. More patients in the normothermic group required low doses of catecholamines, which seems to be associated with vasodilatory effect of normothermia. However, this was not statistically significant. High doses of catecholamines used more often in cold crystalloid cardioplegia group may be explained as negative influence of hypothermia on myocardial function recovery after cardiopulmonary bypass.

Further source of concerns with the use of normothermic bypass was neurological outcome (20,21,25). The incidence of stroke and encephalopathy in our study was the same in all groups, but the number of patients in our study was too small to be conclusive because the incidence of these complications was low.

The main limitation of our study was that the effects of the methods of cardioplegia were investigated within a relatively short period of aorta cross-clamping. We suppose that the differences in the measured parameters would be more pronounced if cardioplegia lasted for two hours or more.

In conclusion, myocardial protection with crystalloid cardioplegia was as good as using blood cardioplegia in stable patients eligible for elective CABG. However, intermittent antegrade warm blood cardioplegia provides better myocardial protection during CABG surgery, which is very important in high-risk patients with poor ventricular function. Further research is necessary to develop the most proper composition of cardioplegic solution, its temperature and delivery to optimize intraoperative myocardial protection.

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