

Myocardial Protection of Warm Cardioplegic Induction on the Isolated Perfused Rat Heart Model

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Abstract: Myocardial protection through different cardioplegia approaches is an important issue for successful cardiovascular surgery. The objective of this prospective randomized study was to evaluate the effect of myocardial protection of warm (37°C) and cold (6°C) cardioplegic induction, respectively, using a Langendorff isolated rat heart perfusion model. Twenty-eight isolated rat hearts on the Langendorff perfusion model were randomly divided into two groups: group T ($n = 14$) received warm (37°C) cardioplegic induction, followed by cold (6°C) cardioplegia after ECG showed straight line; alternatively, group C ($n = 14$) received only cold cardioplegic induction. After undergoing ischemia for 80 min, both group T and group C received reperfusion with Krebs–Henseleit Buffer (KHB) for 40 min. An additional group A ($n = 7$) received KHB continuously for 120 min and served as the control group for the assessment of Na^+/K^+ -ATPase activity. The coronary flow, concentration of creatine kinase (CK) in coronary effluent, and cardiac function were evaluated at different time periods. Na^+/K^+ -ATPase activity was

assessed at the end of reperfusion. The coronary flow, content of CK in coronary effluent, heart rate, $\text{dp}/\text{dt}_{\text{max}}$, and left ventricular peak pressure (LVPP) were significantly greater ($p < .05$) in group T than group C during the reperfusion period. The negative value of $-\text{dp}/\text{dt}_{\text{max}}$ and left ventricular end-diastolic pressure (LVEDP) was significantly lower ($p < .05$) in group T than group C, during the reperfusion period. The Na^+/K^+ -ATPase activity assessed at the end of reperfusion period was significantly higher ($p < .05$) in group A and group T than group C, while no significant difference ($p = .13$) was found between group T and group A. Compared with cold cardioplegic induction, warm cardioplegic induction provides superior myocardial protection by enhancing coronary flow, reducing myocardial injury, improving cardiac function, and preserving Na^+/K^+ -ATPase activity. **Keywords:** cardiovascular surgery, cardiopulmonary bypass, myocardial protection, isolated perfused heart, warm cardioplegic induction. *JECT. 2004;36:58–65*

Achieving optimal myocardial protection through different cardioplegia approaches is critical for successful cardiopulmonary bypass (CPB) during open-heart surgery. Currently, the most popular cardioplegia for adult cardiac surgery in major medical institutions is a mixture of hyperkalemia and oxygenated blood with a ratio of 1:4. This approach can supply oxygen and necessary substrates to the myocardium for maintaining cellular metabolism during myocardial arrest, while avoiding the side effect caused by crystalloid cardioplegia. However, controversy about the optimal temperature of the cardioplegia still

remains, particularly concerning the issue whether cold (4–10°C) or warm (37°C) cardioplegia should be applied (1–4).

We hypothesized that warm blood cardioplegic induction followed by cold cardioplegia maintenance provides better myocardial protection than cold blood cardioplegia alone. In our previous clinical prospective randomized study, we showed that this approach was beneficial to patients undergoing valve replacement, based upon the results of clinical outcomes, serum myocardial injury index (cardiac troponin T), and right atrium biopsy (5). Both IRB and Clinical Administration at Fu Wai Cardiovascular Hospital have accepted this conclusion. The surgeons, anesthesiologists, and perfusionists in this institution have changed the operative procedure in adult patients, from

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cold blood cardioplegia to warm blood cardioplegic induction followed by cold blood cardioplegia maintenance. However, the potential protective mechanism of warm cardioplegic induction remains to be elucidated.

Langendorff et al. reported the isolated heart perfusion model for the first time in 1965 (6). The cardiovascular researchers have since used the Langendorff perfusion model because it provides reliable indices on myocardial function without the influences of nerve and hemodynamic alterations. In this study, we attempted to compare the effects of myocardial protection between warm and cold cardioplegic induction by use of a Langendorff rat heart perfusion model and to reveal the potential mechanisms.

MATERIALS AND METHODS

Langendorff Perfusion Model

All rats used in this study received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). Male Wistar rats (weight 280–320 g, The Jackson Laboratory, Bar Harbor, ME) were anesthetized with sodium pentobarbital (Sigma, St. Louis, MO) 80 mg/kg IP, and then heparinized with heparin sodium (American Pharmaceutical Partners, Los Angeles, CA) 800 u/kg IV through the left femoral vein. The heart was excised and placed in the ice-cold Krebs–Henseleit buffer (KHB, see Table 1), which immediately stopped the contractile activity of the heart. The aorta was rapidly slipped onto a plastic cannula (Powerlab-ADInstruments, Colorado Springs, CO) attached to the perfusion column, and retrograde perfusion was initiated immediately with KHB oxygenated with 95% O₂ and 5% CO₂ at 37°C at a constant perfusion pressure of 75 mmHg. The total time from heart excision to initiation of Langendorff perfusion was less than 1 minute.

An incision was made at the root of pulmonary artery to facilitate coronary effluent drainage. A small latex balloon, which coupled with a graduated threaded microsyringe and a pressure transducer, was inserted into the left ventricular (LV) cavity through a left atrial incision, for

measurements of LV isovolumic pressures. The balloon was inflated with KHB three times until the left ventricular end-diastolic pressure (LVEDP) was maintained at 2 mmHg, to ensure its position in LV cavity. The pressure data were imported and recorded automatically in the CODAS database software recording system.

Isolated Heart Grouping

After a 35 minute stabilization period with the perfusion of KHB, the isolated heart began to receive intermittent cardioplegia (Table 1).

Twenty-eight isolated hearts were randomly divided into two groups: group T ($n = 14$) and group C ($n = 14$). The hearts in group T first received warm cardioplegic induction, followed by cold cardioplegia when the electrocardiogram (ECG) plotted a straight line. Conversely, the hearts in group C received cold cardioplegic induction only. The total amounts of cardioplegic induction in both groups were 15 mL/kg. The cardiac arrest was maintained with intermittent cold cardioplegia (8 mL/kg, 30 min interval), at 6°C in a water-jacket heart chamber for 80 min. Subsequently, the hearts received 40 min reperfusion with KHB at 37°C.

Another seven isolated hearts (group A) received continuous perfusion with KHB for 120 min (80 min + 40 min) at 37°C, to serve as the blank control for the measurement of Na⁺/K⁺-ATPase activity.

Cardiac Mechanical Function

The indices of cardiac function, such as coronary flow rate (CFR), heart rate (HR), cardiac systolic ability (e.g., maximal increased velocity of left ventricular pressure (+dp/dt_{max}), left ventricular peak pressure [LVPP]), and cardiac diastolic ability (e.g., maximal decreased velocity of left ventricular pressure (–dp/dt_{max}), left ventricular end-diastolic pressure [LVEDP]), were recorded at 5 min before cardiac arrest, and 1, 5, 10, 15, 20, 25, and 30 min during KHB reperfusion, respectively.

Measurement of Myocardial Damage

At the same intervals that cardiac function was recorded, and the coronary effluent was collected, the concentration of creatine kinase (CK) in the effluent, which was taken as the index of myocardial damage, was measured with a previously described spectrometric assay (7).

Measurement of Heart Tissue Na⁺/K⁺-ATPase Activity

At the end of 40 min of reperfusion for group T and group C, and at the end of 120 min KHB continuous perfusion for group A, the hearts were cut 100 mg at apex and homogenated. The Na⁺/K⁺-ATPase activity was measured with a previously described spectrometric assay (8).

Table 1. Formulation of Krebs–Henseleit buffer and cardioplegia.

	Krebs–Henseleit Buffer (mmol/L)	Cardioplegia (mmol/L)
NaCl	118.0	118.0
KCl	4.7	20.0
MgSO ₄	1.2	1.2
KH ₂ PO ₄	1.2	1.2
NaHCO ₃	24.9	24.9
CaCl ₂	1.2	1.2
Glucose	11.1	11.1
pH	7.38–7.42	7.38–7.42

Statistical Analysis

MS-SPSS software was used for statistical analysis. Normal distribution data were presented as "mean \pm standard deviation" and received Student's *t*-test. Results were significant when $p < .05$.

RESULTS

Coronary Flow Rate (Figure 1)

Group T remained at the baseline level for 25 min during reperfusion; whereas, group C decreased after 5 min of reperfusion. At all time points during reperfusion, group T was significantly higher than group C.

Heart Rate (Figure 2)

Both group T and group C decreased at the early period of reperfusion. However, group T recovered to baseline (reperfusion 10 min) earlier than group C (reperfusion 30 min).

+dp/dt_{max} (Figure 3)

Both group T and group C decreased at the beginning of KHB reperfusion. Group T recovered to the baseline after 15 min of reperfusion; whereas, group C remained at a significantly lower level. After reperfusion 5 min, group T was significantly higher than group C at all time points.

Left Ventricular Peak Pressure (Figure 4)

Group T declined at the beginning of reperfusion, and then recovered to the baseline level after reperfusion for 15 min. Group C did not recover to the baseline during reperfusion. At all time points of reperfusion period, group T was significantly higher than group C.

-dp/dt_{max} (Figure 5)

Group T increased at reperfusion of 1 min and 5 min; decreased back to baseline at reperfusion of 10 min and 15 min, and then increased again from reperfusion after 20 min. Group C increased at the beginning of reperfusion and remained at a higher level than group T during the reperfusion time periods.

Left Ventricular End-Diastolic Pressure (Figure 6)

Both group T and group C increased at the beginning of reperfusion, and then decreased slowly without reaching the baseline. After reperfusion for 10 min, group T was much lower than group C.

Creatine Kinase (CK) Concentration in Coronary Effluent (Figure 7)

Both group T and group C initially increased after reperfusion; however, they began to decline after 1 min of reperfusion. Group T recovered to the baseline after reperfusion for 10 min; whereas, group C did not reach the baseline. At all points during reperfusion, group T was significantly lower than group C.

Na⁺/K⁺-ATPase Activity (Figure 8)

At 40 min of reperfusion for group T and group C and 120 min perfusion for group A, group A and group T was significantly higher than group C, and no significant difference was found between group A and group T.

DISCUSSION

Currently, a number of cardiovascular surgeries are supported by cardiopulmonary bypass with a period of cardiac arrest. Intermittent (30 min interval) cold (4–8°C)

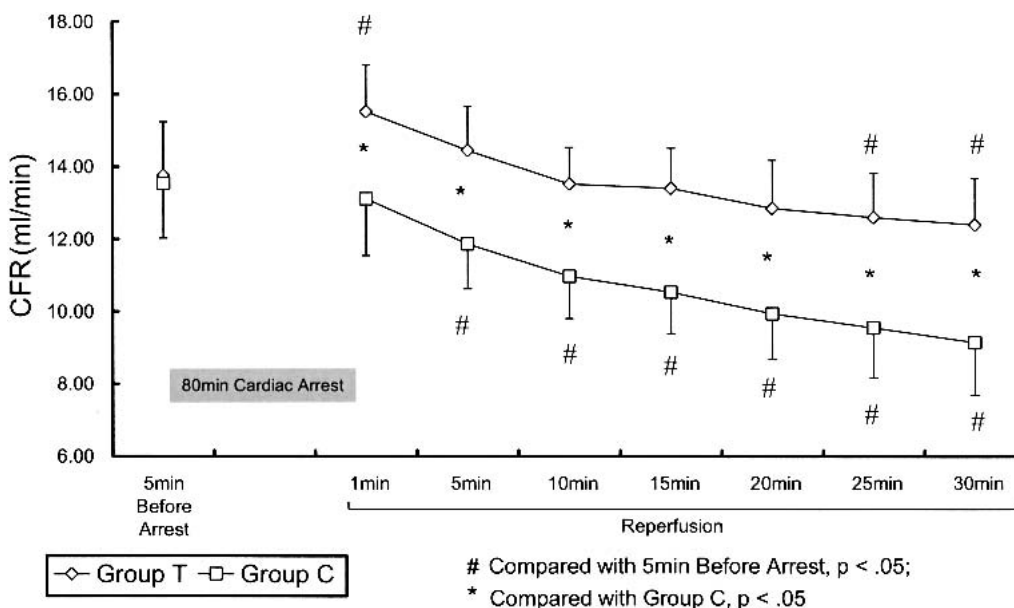


Figure 1. Coronary flow rate (CFR, milliliter per minute).

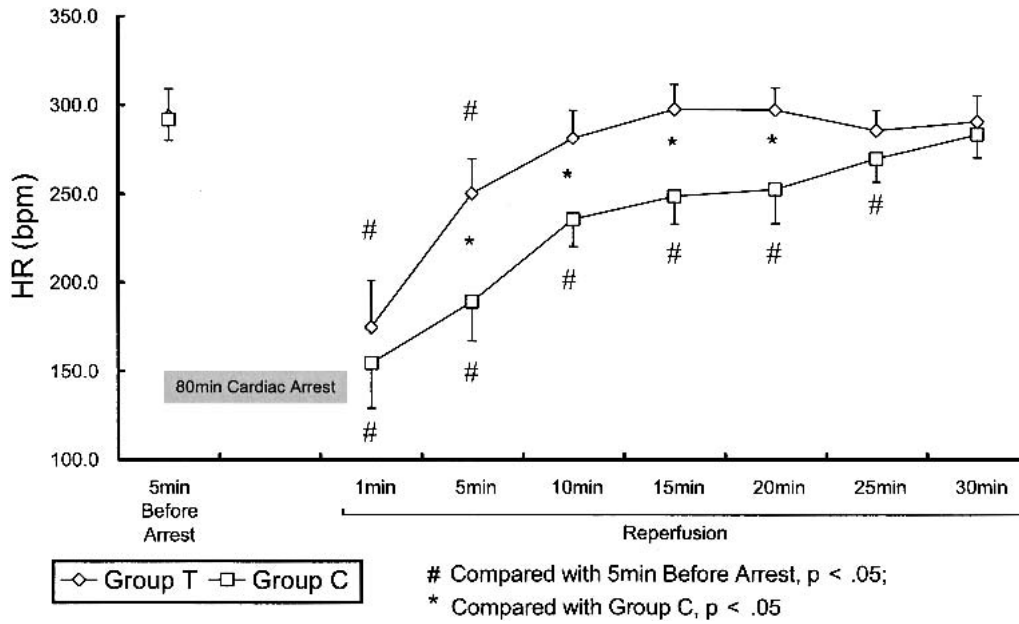


Figure 2. Heart rate (HR, beats per minute).

blood cardioplegia is the most popular approach for adult patients. The advantages of this approach are the following: providing a quiet and clear surgical field, reducing myocardial metabolism, providing necessary substratum and oxygen of phosphorylation, and therefore, attenuating ischemia and reperfusion injury.

Recently, some studies demonstrated that cold blood cardioplegia, especially cold blood cardioplegic induction on the first-time delivery, inevitably produced side effects, some of which were severe. Cold cardioplegic induction leads to lower enzymatic activity, injured biomembrane structure, "cold contracture" of cardiomyocyte, obstruc-

tion of coronary capillary, maldistribution of cardioplegia, and injury of blood cells (9–11). Therefore, the conception of "warm heart surgery" was developed (12). The warm heart surgery, through which continuous warm blood cardioplegia is used during cardiopulmonary bypass, is supported by the theory that the metabolic rate reduces 90%, while the cardiac electric and mechanical activities are arrested. Therefore, continuous low flow rate of hyperkalaemic blood cardioplegia could provide sufficient oxygen and substrate (13). However, the clinical application of this approach with warm CPB and continuous blood cardioplegia is limited to the operations in cardiac surface

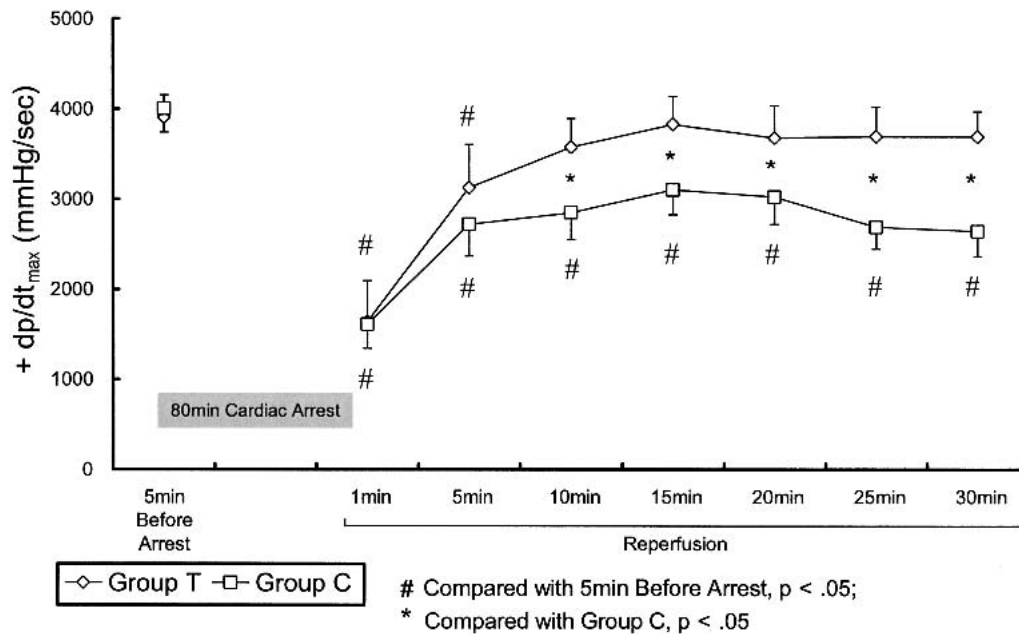


Figure 3. Maximal increased velocity of left ventricular pressure (+dp/dt_max, mmHg per second).

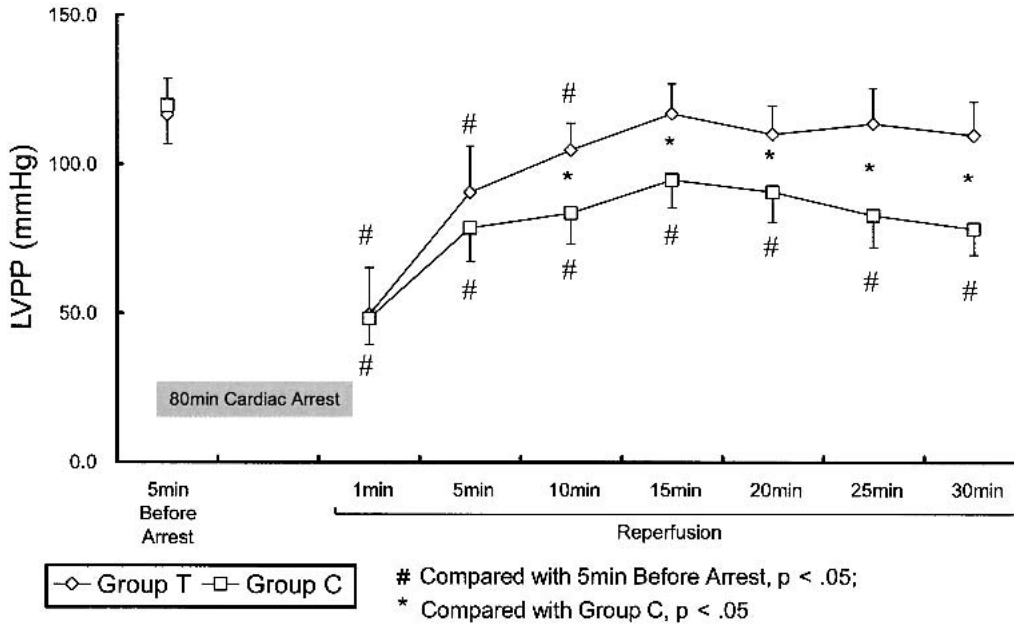


Figure 4. Left ventricular peak pressure (LVPP, mmHg).

procedures (e.g., coronary artery bypass grafting), because standard warm heart surgery could not provide clean operative fields for surgeons in open-heart procedures (e.g., valve replacement and septal defect repair). Therefore, some investigators considered warm blood cardioplegic induction to get the advantages of both warm and cold cardioplegia. First, patients receive warm cardioplegia, and then, as soon as the ECG monitor graphs a straight line, they receive cold blood cardioplegia until the amount of induced cardioplegia reaches 15–20 mL/kg. Cold blood

cardioplegia is used as subsequent intermittent (every 30 min) maintenance (5,14,15).

In this prospective randomized study, oxygenated Krebs–Henseleit buffer and cardioplegia were used to simulate clinical arterial blood flow and blood cardioplegia. Our results support the notion that, warm cardioplegic induction, compared with cold cardioplegic induction, provides better myocardial protection.

Under the constant pressure (75 mmHg) perfusion model, the coronary flow rate (CFR) is determined largely

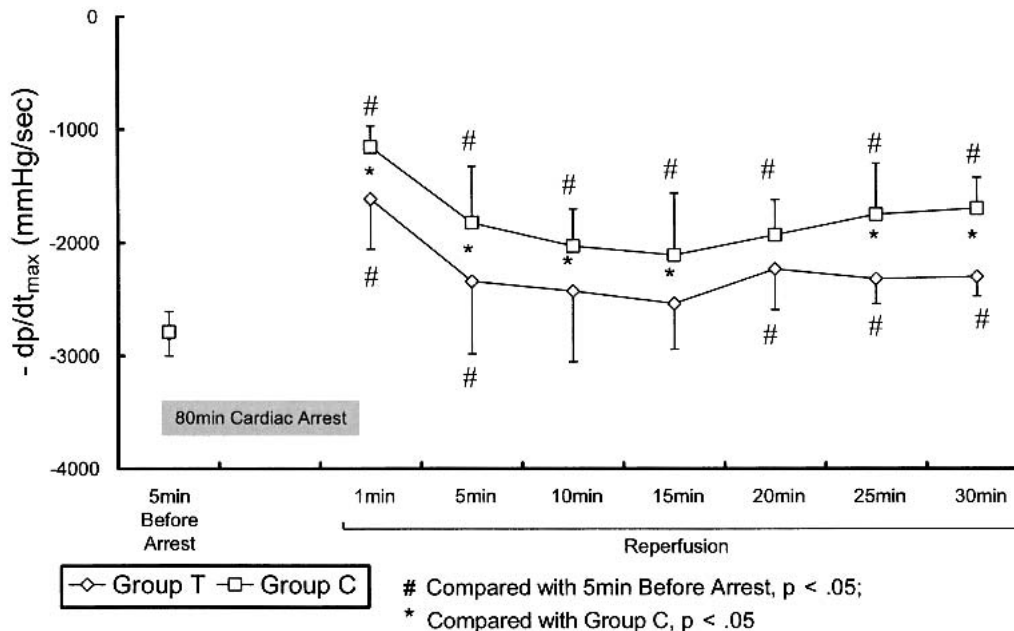


Figure 5. Maximal decreased velocity of left ventricular pressure ($-dp/dt_{max}$, mmHg per second).

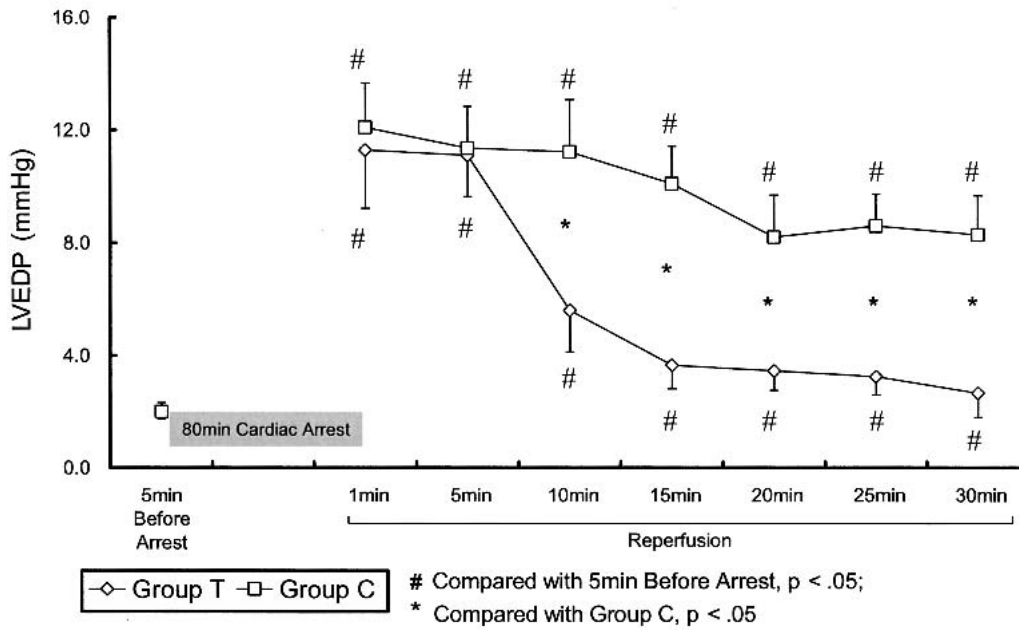


Figure 6. Left ventricular end-diastolic pressure (LVEDP, mmHg).

by the coronary circulatory resistance, which depends on the functional status of microarterial smooth muscle and capillary endothelium. The coronary flow rate showed an increased tendency on coronary resistance during reperfusion, indicating that the smooth muscle cells and endothelial cells might be injured during ischemia and reperfusion (see Figure 1). The higher levels of CFR in group T during reperfusion indicated that warm cardioplegic induction could provide better protection on coronary circulation than cold cardioplegic induction.

The electrophysiological character of cardiomyocytes includes the irritability, rhythmicity, and conductivity. The general appearance of the electrophysiological function on global heart is presented as the heart rate and rhythm. The heart rate of an isolated rat heart varies between 260–320 bpm under physiological condition, and becomes lower with the myocardial injury. In our present study, the heart rates of both group T and group C decreased significantly at the early period of reperfusion, and increased gradually, indicating the ischemia and re-

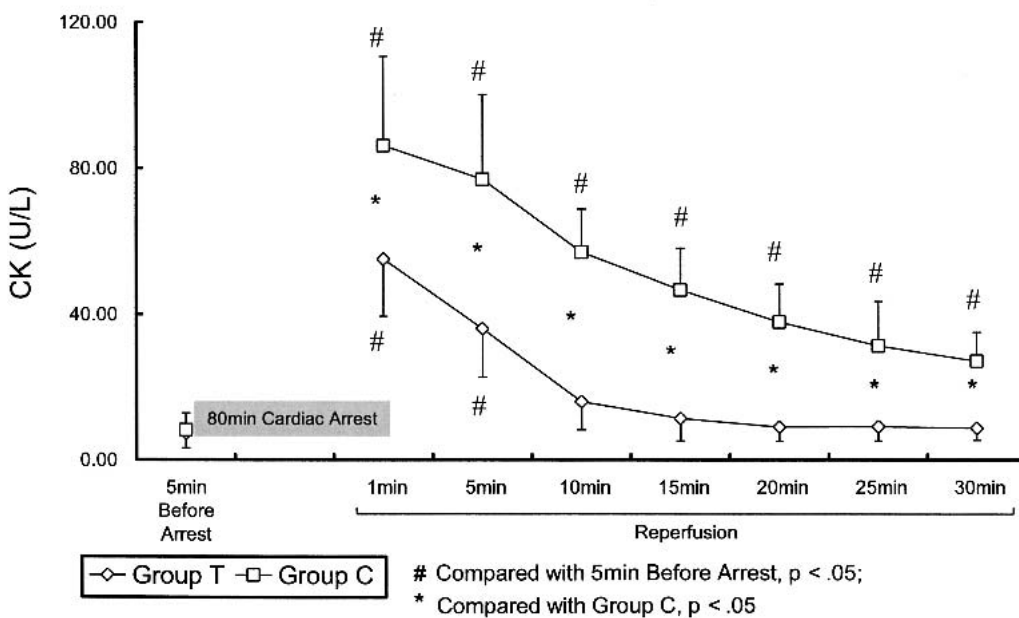


Figure 7. Creatine kinase concentration in coronary effluent (CK, units per liter).

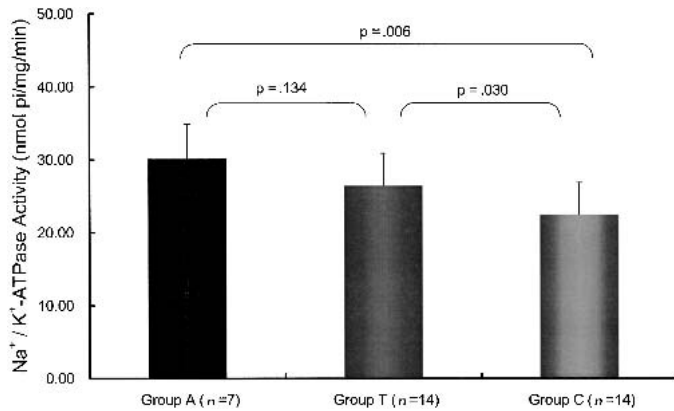


Figure 8. Na⁺/K⁺-ATPase activity (Na⁺/K⁺-ATPase, nmol pi per milligram per minute).

perfusion injury. The heart rate in group T recovered to baseline (10 min) earlier than that in group C (30 min) and was much higher than that in group C during the early periods of reperfusion (see Figure 2), indicating that warm cardioplegic induction could attenuate the electrophysiological damage of cardiomyocytes.

In the isolated perfused hearts, the major indices of systolic character are the maximal increased velocity of left ventricular pressure (+dp/dt_{max}), and left ventricular peak pressure (LVPP); the major indices of diastolic character are the maximal decreased velocity of left ventricular pressure (-dp/dt_{max}), and left ventricular end-diastolic pressure (LVEDP). The results of +dp/dt_{max} and LVPP showed that the systolic function of both group T and group C decreased significantly at the early period of reperfusion and increased gradually later. The systolic function of group T recovered to baseline at reperfusion 15 min; whereas, that of group C did not recover during reperfusion and showed a decreasing tendency again at the same time point (see Figures 3 and 4). The results of -dp/dt_{max} and LVEDP showed that the diastolic function of both group T and group C decreased during reperfusion and did not recover to baseline, indicating that the diastolic function was damaged severely and could not recover in a short time (Figures 5 and 6). The results of systolic and diastolic indices suggested that warm cardioplegic induction could protect the cardiac mechanical function better than cold cardioplegic induction.

CK exists in the cytoplasm of cardiomyocyte and will be released out as soon as the myocardium is damaged. Although cardiac CK presents some cross reaction with skeletal muscle, the CK contained in coronary effluent in this study indicated the damage of cardiomyocytes because of the isolated heart. The results of CK showed that CK content of both group T and group C reached a peak value at the very beginning of reperfusion and then decreased. CK in group T recovered to baseline at reperfusion 15 min; whereas, CK in group C did not go back to baseline, and furthermore, was higher significantly than that in

group T at all time points during reperfusion (Figure 7). The results of CK indicated that warm cardioplegic induction could attenuate the ischemia and reperfusion injury to the cardiomyocytes.

Na⁺/K⁺-ATPase; namely, Na⁺/K⁺ pump, is one of the most important energy-dependent ion channels, to modulate the ion exchange, and to maintain the action potential and cellular osmotic pressure between the two sides of membrane. The measurement of Na⁺/K⁺-ATPase activity reflects the disturbance of high-energy phosphate. In this study, at the end of (re)perfusion, the sequence of Na⁺/K⁺-ATPase activity is group A, group T, and group C (see Figure 8), suggesting that myocardial ischemia might reduce the production of adenosine triphosphate (ATP), and, therefore, lower the activity of energy-dependent enzymes, and warm cardioplegic induction could attenuate this damage.

In summary, compared with cold cardioplegic induction, warm cardioplegic induction provides superior myocardial protection, by enhancing coronary flow, improving cardiac electrophysiological and mechanical function, attenuating ischemia and reperfusion injury, and preserving energy-dependent enzyme activity.

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