Original Article

Retrograde Continuous Warm Blood Cardioplegia Reduces Oxidative Stress during Coronary Artery Bypass Grafting

Noritsugu Morishige MD, Tadashi Tashiro MD, Takashi Yamada MD, and Michio Kimura MD

Myocardial oxidative stress during retrograde continuous blood cardioplegia (RCBC) was evaluated in 22 patients undergoing elective aortocoronary bypass surgery. The patients were divided into two groups: Group C (n=11) received cold RCBC, and Group W (n=11) received warm RCBC. Myocardial oxidative stress was assessed by measuring the release of oxidized glutathione (GSSG), malondialdehyde (MDA), and myeloperoxidase (MPO) in the coronary sinus plasma before aortic clamping, at 1, 5, and 10 minutes after unclamping. Both the hemodynamic recovery and the creatine kinase MB (CKMB) activity were measured perioperatively until 24 hours after unclamping. In Group C, a significant coronary sinus release of GSSG was found in the early reperfusion period in comparison to Group W. No significant difference in the release of MDA nor MPO was noted in the two groups. The recoveries in the left and right ventricular functions, and the peak CK-MB activity were similar in both groups. In conclusion, warm blood cardioplegia is thus considered to protect the myocardium from ischemia-reperfusion injury better than cold blood cardioplegia under retrograde continuous perfusion. (Ann Thorac Cardiovasc Surg 2002; 8: 31–37)

Key words: oxidative stress, glutathione, ischemia, reperfusion, warm blood cardioplegia.

Introduction

The retrograde administration of blood cardioplegia through the coronary sinus is an effective method for avoiding the maldistribution of cardioplegic solutions associated with the antegrade delivery of cardioplegia when either an acute or critical obstruction of coronary arteries exist.[1,2] Despite this benefit, a malperfusion of the posterior septum and right ventricle has been reported during retrograde delivery in experimental studies.[3] Currently, there is increasing interest in the use of normothermic blood cardioplegia. The continuous administration of warm blood cardioplegia produces good myocardial preservation, resuscitates the ischemic myocardium, and reduces both morbidity and mortality in high-risk patients.[4] The retrograde administration of normothermic blood cardioplegia may resuscitate the ischemic myocardium in cases of an acute obstruction of the anterior descending coronary artery, but may provide limited protection of the posterior septum and right ventricle, and also cause ischemic damage in these areas.[5-7] Ischemia or reperfusion injuries may increase under normothermic conditions, but the optimal temperature of retrograde blood cardioplegia remains controversial.[8]

The generation of free radicals and oxidative stress, which induces myocardial injuries in the ischemia-reperfusion period,[9] and the detection of oxidative stress after cardioplegic arrest are sensitive indicators of the efficacy of myocardial protection.

In the present study, we evaluated the safety and efficacy of retrograde continuous warm blood cardioplegia by detecting myocardial oxidative stress and evaluating the postoperative myocardial function in 22 patients undergoing elective coronary artery bypass grafting (CABG) operations using retrograde continuous blood cardioplegia (RCBC), at warm and cold temperatures.

From the Department of Cardiovascular Surgery, University of Fukuoka School of Medicine, Fukuoka, Japan

Received June 4, 2001; accepted for publication August 2, 2001.
Address reprint requests to Noritsugu Morishige, MD: Department of Cardiovascular Surgery, University of Fukuoka School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.

Materials and Methods

Patient population

Twenty-two patients undergoing elective CABG at our institute from May through October 1955 enrolled in this study. Eleven patients, Group C, received continuous retrograde cold blood cardioplegia, and another 11 patients, Group W, received continuous retrograde warm blood cardioplegia. All procedures were performed by the same surgeon (T.T.) of our group. This study complied with the ethical guidelines of our university, and informed consent was obtained from all patients.

Surgical technique

Cardiopulmonary bypass was instituted with a single two-stage right atrial cannula, and an ascending aortic cannula, at a flow rate of 2.2-2.8 liter·min^{-1}·m^{-2}. Mild hypothermia was induced to maintain a 34°C rectal temperature. Blood cardioplegia was prepared by mixing four parts of oxygenated blood with a crystalloid additive, modified Frames' solution (500 ml of 5% dextrose in water, 100 ml of saline, 3 mEq THAM, 4 mEq $MgSO_4$, 10 ml of CPD solution, 18 or 60 mEq KCl), using a Buckberg-Shiley BCP delivery system (BCD-PLUS, Shiley Incorporated, Irvine, CA). A high potassium cardioplegic solution was initially administered into the aortic root at an aortic pressure of 80mmHg to induce cardiac arrest. A low potassium cardioplegic solution was subsequently infused into the coronary sinus continuously at a flow rate of 100 to 200 ml/min at a pressure of 20-40 mmHg. During distal anastomosis, blood cardioplegia was interrupted at short intervals (<10 min) to facilitate visualization. Blood cardioplegia was administered at a temperature of 20°C in Group C (cold blood cardioplegia), and 37°C in Group W (warm blood cardioplegia) by the Shiley BCP delivery system. In both groups, terminal warm blood cardioplegia was infused retrogradely at a flow rate of 200-250 ml/min for 5 minutes before aortic crossclamp removal.

Coronary sinus sampling

Both the myocardial metabolism and oxidative stress were assessed before aortic cross clamping, at 1, 5 and 10 minutes after removal of the crossclamp, by sampling the blood simultaneously from the arterial line and the coronary sinus. All samples were analyzed for the presence of reduced glutathione (GSH), oxidized glutathione (GSSG), lipid peroxides, leukocyte activation, blood gases, and lactate. GSH and GSSG were assessed according to the method by Tietze[10] and modified by Adams et al.[11] Lipid peroxidation was evaluated by assay of thiobarbituric acid reactive substance (TBARS). TBARS were measured by the method by Uchiyama and Mihara,[12] and expressed as malondialdehyde (MDA). Neutrophil activation was assessed by measuring serum myeloperoxidase level using the ELISA method (MPO-EIA, BIOXYTECH S.A., BONNEUIL/MARNE, FRANCE). The myocardial metabolism was analyzed by measuring oxygen tension (PO_2), carbon dioxide tension, pH (Acid-Base Laboratory model 530, Radiometer, Copenhagen), hemoglobin (Hb) and oxygen saturation (SO_2) (Hemoximeter OSM3, Radiometer, Copenhagen), and lactate (YSI model 23L lactate analyzer, Yellow Springs Instrument Co., Ohio). The myocardial oxygen extraction ratio (O_2EXR) was calculated by the following formula: O_2 EXR = (oxygen content of arterial blood – oxygen content of venous blood) / oxygen content of arterial blood. The oxygen content (O_2Cont) was calculated by the following formula: $O_2Cont = 1.34 \cdot Hb \cdot SO_2 + 0.003 \cdot PO_2$. The myocardial lactate extraction ratio (LacEXR) was calculated by the following formula: LacEXR = (lactate concentration of arterial blood – lactate concentration of venous blood) / lactate concentration of arterial blood.

Creatine kinase

Serial blood samples for creatine kinase-MB (CKMB) were obtained postoperatively at 0, 1, 3, 6, 12, and 24 hours, and then were analyzed by the antibody inhibition method (Monotest CKMB, Boreinger Mannheim).

Hemodynamic assessment

Hemodynamic data were obtained using a radial arterial catheter and a thermodilution pulmonary catheter with a rapid thermistor (Baxter Healthcare Corp., Irvin, CA). The outputs from the thermodilution catheter were interfaced to a thermodilution ejection fraction computer (Explorer; Baxter Healthcare Corp., Irvin, CA), RV ejection fraction (RVEF), cardiac output, and RV end-diastolic volume were computed from the thermodilution curve. The mean arterial pressure (MAP), pulmonary capillary wedge pressure (PCWP), mean pulmonary arterial pressure (PAP), and mean right atrial pressure (RAP) and heart rate (HR) were also measured. The derived hemodynamic indices were calculated as follows: Cardiac index (CI) = cardiac output/body surface area (L·min^{-1}·m^{-2}); the left ventricular stroke work index (LVSWI) = CI·HR^{-1}·(MAP–PCWP)·0.0136 (g·m·min^{1}·m^{-2}); the right ventricular

Table 1. Preoperative demographics of the patient groups

	Group C	Group W	p value
Patient number	11	11	
Sex (male / female)	9 / 2	10 / 0	NS
Age (years)	67.1 ± 1.6	63.3 ± 2.4	NS
NYHA functional class	2.5 ± 0.2	2.2 ± 0.3	NS
LVEF (%)	66.0 ± 1.8	63.6 ± 5.0	NS
LVEDP (mmHg)	11.9 ± 1.8	10.4 ± 1.8	NS
History of MI	5	6	NS
Number of diseased Vessels	2.6 ± 0.2	2.6 ± 0.2	NS
LMT lesion	3	1	NS

Where applicable, the values are given as the mean ± standard error of the mean; NYHA: New York heart association; LVEF: left ventricular ejection fraction; MI: myocardial infarction; LMT: left main trunk lesion >75%; NS: not significant.

Table 2. Operative demographics and results

	Group C	Group W	p value
XCL time (min)	51.4 ± 4.4	67.1 ± 4.9	p<0.05
CP interruption (min)	4.8 ± 1.8	5.2 ± 1.9	NS
CP temperature (°C)	20.4 ± 0.6	36.2 ± 1.3	p<0.001
Mean CP flow rate (ml/min)	177.1 ± 9.1	198.2 ± 6.9	NS
Lowest rectal temperature (°C)	33.9 ± 0.3	33.9 ± 0.4	NS
Number of grafts per patient	2.6 ± 0.2	3.6 ± 0.3	p<0.05
Perioperative MI*	0	0	NS
Perioperative IABP	1 (9.0%)	0	NS
Spontaneous defibrillation	9 (81.8 %)	10 (90.9%)	NS
Used dose of CA (mg/kg)#	8.8 ± 1.5	8.4 ± 0.9	NS
Operative mortality	0	0	NS

Where applicable, the values are given as the mean ± standard error of the mean; XCL: cross-clamping; CP: cardioplegia; * peri-operative myocardial infarction (CK-MB max > 100IU/L and new Q wave); # total amounts of catecholamine (dopamine and dobutamine) given during the first 24 hours of reperfusion; NS: not significant.

stroke work index (RVSWI) = $CI \cdot HR^{-1} \cdot (PAP–RAP) \cdot 0.0136$ ($g \cdot m \cdot min^{1} \cdot m^{-2}$). All measurements were recorded before a sternotomy, and at 1, 3, 6, 12, and 24 hours after the termination of cardiopulmonary bypass.

Statistics

All data are presented as the mean ± SEM. The preoperative clinical variables were compared by unpaired t-tests or an χ^2 analysis. Postoperative hemodynamic and biochemical parameters were compared by one-way and two-way repeated measures ANOVA. A p value less than 0.05 was considered to be statistically significant.

Results

No significant difference was seen in the preoperative profiles between the two groups as shown in Table 1. The number of grafts per patient and the cross clamping time was longer in the warm group (p<0.05, Table 2). The mean infusion flow rate and interruption time of the continuous cardioplegic solution during cross clamping was similar in both groups. The clinical outcomes are shown in Table 2. No hospital death occurred in either group. No patients had a perioperative myocardial infarction defined as either new Q waves and rises in CK-MB levels higher than 100 IU/l. One patient in the Group C required intraaortic balloon pump support after operation. The frequency of spontaneous electromechanical activity was similar in the two groups. There was no difference in the dose of inotropic agents required during the first 24 hours after cross clamp removal. The hemodynamic performances before and after reperfusion were similar in the

Table 3. Summary of the cardiac functional parameters and the plasma CKMB levels before and after cross clamping.

	PRE	XCL off 1 hour	XCL off 3 hours	XCL off 6 hours	XCL off 12 hours	XCL off 24 hours
Group C						
HR (bpm)	67.8 ± 4.3	107.1 ± 3.7	95.3 ± 5.6	93.4 ± 4.8	84.3 ± 3.7	82.6 ± 3.0
PCWP (mmHg)	9.9 ± 0.8	10.0 ± 1.4	7.3 ± 0.5	8.3 ± 1.0	8.1 ± 0.8	9.3 ± 0.4
CI (L/min/m^2)	3.3 ± 0.3	4.1 ± 0.2	3.2 ± 0.4	3.1 ± 0.4	2.9 ± 0.3	3.1 ± 0.4
LVSWI (g m/m^2)	54.4 ± 3.9	34.9 ± 3.0	33.9 ± 4.1	33.6 ± 3.9	34.7 ± 3.8	38.9 ± 4.0
RVSWI (g m/m^2)	7.9 ± 1.1	6.3 ± 0.9	4.8 ± 1.0	5.6 ± 0.8	4.7 ± 0.6	5.1 ± 0.7
RVEF (%)	38.2 ± 1.8	37.5 ± 2.2	34.3 ± 1.2	31.8 ± 1.5	29.6 ± 2.3	34.0 ± 1.8
CK-MB (IU/L)	2.08 ± 0.63	19.96 ± 1.72	20.09 ± 1.88	22.92 ± 2.67	19.33 ± 3.84	14.01 ± 2.46
Group W						
HR (bpm)	63.5 ± 3.9	107.9 ± 7.8	102.8 ± 5.6	99.9 ± 3.6	89.4 ± 3.9	86.6 ± 2.8
PCWP (mmHg)	10.5 ± 1.0	10.4 ± 1.3	9.2 ± 1.4	10.6 ± 1.4	11.6 ± 0.9	11.3 ± 0.7
CI (L/min/m^2)	3.1 ± 0.2	4.3 ± 0.3	3.6 ± 0.3	4.0 ± 0.2	3.3 ± 0.2	3.5 ± 0.2
LVSWI (g m/m^2)	52.0 ± 3.5	33.1 ± 3.7	33.9 ± 3.4	37.1 ± 1.8	30.8 ± 1.7	37.0 ± 1.9
RVSWI (g m/m^2)	6.7 ± 0.8	5.0 ± 1.0	4.8 ± 0.8	6.4 ± 0.7	4.1 ± 0.5	4.5 ± 0.6
RVEF (%)	38.4 ± 0.9	34.6 ± 2.6	34.8 ± 1.8	33.7 ± 1.6	32.3 ± 1.9	32.3 ± 2.2
CK-MB (IU/L)	2.40 ± 1.03	22.51 ± 2.38	22.96 ± 2.18	19.51 ± 1.68	18.61 ± 4.64	11.86 ± 2.65

Values are given as the mean ± standard error of the mean; HR, PCWP, CI, LVSWI, RVSWI, RVEF and CKMB were not significantly different between the groups (repeated measures ANOVA); HR: heart rate; PCWP: pulmonary capillary wedge pressure; CI: cardiac index; LVSWI: left ventricular stroke work index; RVSWI: right ventricular stroke work index; RVEF: right ventricular ejection fraction.

Table 4. Myocardial metabolism before cross clamping and during reperfusion

	Group C	Group W
EXR-O_2		
Before XCL	0.43 ± 0.03	0.46 ± 0.02
XCL off 1 min	0.18 ± 0.01	0.19 ± 0.01
5 min	0.38 ± 0.04	0.37 ± 0.02
10 min	0.43 ± 0.04	0.52 ± 0.02
EXR-Lac		
Before XCL	–0.07 ± 0.06	–0.05 ± 0.06
XCL off 1 min	0.06 ± 0.02	0.07 ± 0.02
5 min	0.08 ± 0.02	0.13 ± 0.02
10 min	–0.01 ± 0.08	0.05 ± 0.04

EXR-O_2: myocardial oxygen extraction ratio; EXR-Lac: myocardial lactate extraction ratio; XCL: cross clampimg; XCL off: cross clamp removal; EXR-O_2 were not significantly different between the groups (p=0.2012, repeated measures ANOVA); EXR-Lac were not significantly different between the groups (p=0.3842, repeated measures ANOVA).

two groups as shown in Table 3. Neither group had any significant difference in the serum CK-MB level during reperfusion (Table 3). Both myocardial oxygen extraction ratio and lactate extraction ratio during reperfusion were similar in the two groups (Table 4). Myocardial oxidative stress during reperfusion was shown in Fig. A significant coronary sinus release of GSH was found in Group C in comparison to Group W (p=0.0204, repeated measures ANOVA). A significant coronary sinus release of GSSG was found in Group C in comparison to Group W (p=0.0088, repeated measures ANOVA). The production of lipid peroxidation products (MDA) was observed at an early period of reperfusion in Group C, but the difference was not statistically significant. Neutrophil activation assessed by measuring the serum myeloperoxidase level was also shown in Fig. A significant increase in the arterial myeloperoxidase level was found in both groups after reperfusion, but no transmyocardial release of myeloperoxidase was evident in either group.

Discussion

The oxygen radicals have been suggested to induce deleterious alterations and affect the functional and metabolic recovery in reperfused human hearts.[13] To counteract the oxygen radicals, the cells are provided with scavenging systems. A major defense against reactive oxygen radicals is the glutathione redox cycle. Oxidants are inactivated by the reaction of reduced glutathione (GSH) with the formation of oxidized glutathione (GSSG). When the cells are exposed to a large amount of oxidants, the rate of GSSG formation may exceed the rate of reducing GSSG to GSH, thus resulting in oxidative stress. Accumulated GSSG is transported out of the cell, and GSSG

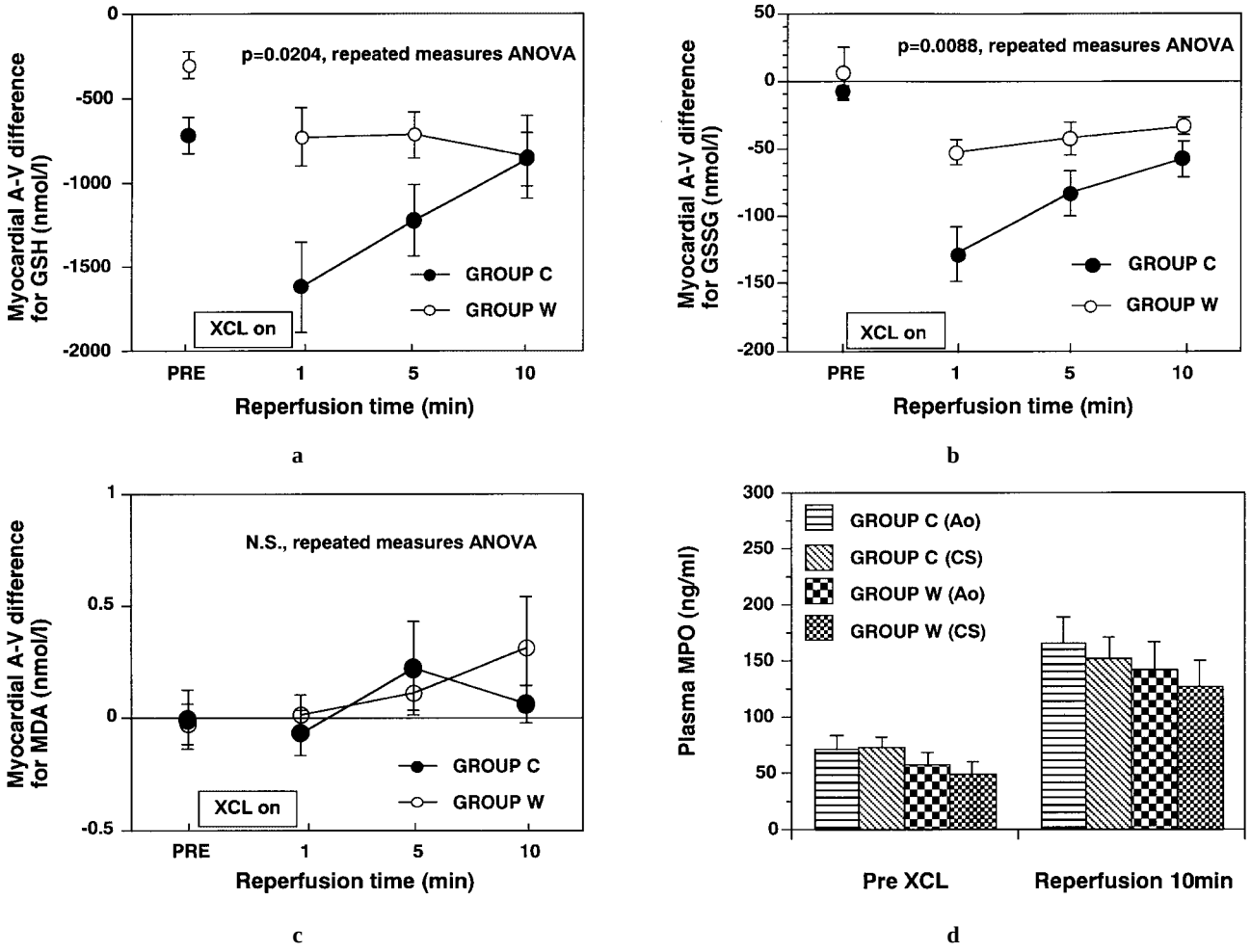

Fig. Myocardial arterial-coronary sinus difference for (a) the plasma reduced glutathione (GSH) levels, (b) the plasma oxidized glutathione (GSSG) levels, (c) the malondialdehyde (MDA) levels, and (d) the myeloperoxidase (MPO) levels before and after cross clamping removal in patients undergoing coronary bypass surgery. All values are expressed as the mean ± standard error of the mean. The groups were significantly different in GSH (p=0.0204, repeated measures ANOVA), and GSSH (p=0.0088, repeated measures ANOVA).

efflux is linearly related to the intracellular concentrations of GSSG over a wide range.[9,14] As a result, the increased release of GSSG is a sensitive and specific index of intracellular oxidative stress. Moreover, the coronary sinus release of GSH is an important marker of myocyte membrane dysfunction.[15] This study shows that warm retrograde blood cardioplegia significantly reduced the coronary sinus release of GSSG and GSH in comparison to that of cold retrograde blood cardioplegia. These findings suggest that warm blood cardioplegia protects the myocardium and reduces the myocardial oxidative stress and myocyte membrane damage during reperfusion even under retrograde delivery. We also found no significant differences in left and right ventricular function and catecholamine requirement after surgery, no significant differences in the serum CK-MB level were observed after surgery which reflected the existence of myocardial damage in both groups. These results suggest that the normothermic retrograde delivery of blood cardioplegic solution protects both sides of the heart during cross-clamping for cold blood cardioplegia. A recent study demonstrated that all regions of both ventricles were perfused by retrograde cardioplegia in an excised human heart.[16] Furthermore, intraoperative contrast echocardiography in the human heart had demonstrated a homogeneous distribution of retrograde cardioplegia to the region of both ventricles.[17] This discrepancy between the experimental observations and favorable clinical results of retrograde warm blood cardioplegia is explained by the differences in the coronary venous anatomy of both human hearts

and canine hearts.[3,16]

The protective effect of hypothermia on cardioplegic arrest remains controversial. Hypothermia inhibits mitochondrial respiration, oxidative phosphorylation, and glycolysis, and reduces ATP regeneration.[18] Glutathione reductase regenerates GSH using reduced equivalents from a NADPH generated by a hexose monophosphate shunt,[19] and the regeneration of GSH is reduced as a consequence of a decrease in the available NADPH under hypothermia. This is one possible mechanism for the presence of enhanced oxidative stress in the cold blood cardioplegia group. Another antioxidant system is the endogenous free radical scavengers of the blood cardioplegic solution. Intact red cells have the ability to scavenge extracellularly generated hydrogen peroxide, and to inhibit the formation of hydroxyl radicals and hypochlorous acid.[20] Intact erythrocytes are able to decrease the endogenously generated hydrogen peroxide and related reperfusion injury in isolated perfused rat hearts, and the scavenging mechanism of the erythrocyte involved in the inactivation of hydrogen peroxide by catalase and glutathione within the erythrocytes.[21] The antioxidant capacity of erythrocytes is almost entirely due to intracellular catalase, and its activity is temperature dependent. This further supports the theory that warm blood cardioplegia has a superior inhibitory effect on oxidative stress during reperfusion.

Hypothermia adversely affects oxygen delivery to the tissue with a shift of the oxyhemoglobin dissociation curve,[22] and with a cold induced vasoconstriction. A decreased oxygen transport renders the tissue hypoxic or ischemic, and an oxygen free radical is generated during reperfusion or reoxygenation. Oxygen free radical production during the reperfusion period is suggested to cause postoperative myocardial dysfunction after cardioplegic arrest.[9] Kukreija and associates demonstrated that the oxygen radicals oxidized thiol groups in the SR enzymes and thus impaired the SR functions.[23] Liu and associates demonstrated that the activity of sarcoplasmic reticulum Ca^{2+}-ATPase was better preserved after reperfusion in warm blood cardioplegia than in cold blood cardioplegia.[24] These findings provide evidence for an improved functional recovery after warm blood cardioplegia. Enhanced oxidative stress after cold cardioplegia may thus impair the SR function and depress the systolic and diastolic function after reperfusion. A continuous perfusion of the normothermic blood cardioplegia could help maintain the mitochondrial function, enzyme activity of the antioxidant enzymes, reduce oxidative stress during the reperfusion period, and thereby improve the post ischemic functional recovery. Despite the fact that oxidative stress (GSSG production) and myocardial membrane damage (GSH release) were significantly observed in the cold cardioplegia group, no differences in either the functional recovery or myocardial CK-MB release were consistently demonstrated in our present study. The relatively short cross-clamping time and the combination of terminal warm blood cardioplegia even in the cold blood cardioplegia group may thus have caused this discrepancy.

In conclusion, the retrograde perfusion of warm blood cardioplegia safely protects the human myocardium as cold blood cardioplegia. Furthermore, warm blood cardioplegia reduces the degree of myocardial oxidative stress during the reperfusion period after the use of cold blood cardioplegia. This effect may help protect and resuscitate the myocardium under conditions of more severe or prolonged ischemia.

References

1. Partington MT, Acar C, Buckberg GD, Julia P, Kofsky ER, Bugyi HI. Studies of retrograde cardioplegia. I. Capillary blood flow distribution to myocardium supplied by open and occluded arteries. *J Thorac Cardiovasc Surg* 1989; **97**: 605–12.
2. Noyez L, van Son JAM, van der Werf T, et al. Retrograde versus antegrade delivery of cardioplegic solution in myocardial revascularization. *J Thorac Cardiovasc Surg* 1993; **105**: 854–63.
3. Shiki K, Masuda M, Yonenaga K, Asou T, Tokunaga K. Myocardial distribution of retrograde flow through the coronary sinus of excised normal canine heart. *Ann Thorac Surg* 1986; **41**: 265–71.
4. Lichtenstein SV, Ashe KA, El Dalati H, Cusimano RJ, Panos A, Slutsky AS. Warm heart surgery. *J Thorac Cardiovasc Surg* 1991; **101**: 269–74.
5. Menasché P, Fleury JP, Droc L, et al. Metabolic and functional evidence that retrograde warm blood cardioplegia does not injure the right ventricle in human beings. *Circulation* 1994; **90** (part II): 310–5.
6. Hayashida N, Ikonomidis JS, Weisel RD, et al. Adequate distribution of warm cardioplegic solution. *J Thorac Cardiovasc Surg* 1995; **110**: 800–12.
7. Allen BS, Winkelmann JW, Hanafy H, et al. Retrograde cardioplegia dose not adequately perfuse the right ventricle. *J Thorac Cardiovasc Surg* 1995; **109**: 1116–26.
8. Hayashida N, Weisel RD, Shirai T, et al. Tepid antegrade and retrograde cardioplegia. *Ann Thorac Surg* 1995; **59**: 723–9.
9. Ferrari R, Alfieri O, Curello S, et al. Occurrence of oxidative stress during reperfusion of human heart. *Circulation* 1990; **81**: 201–11.
10. Tietze F. Enzymatic method for quantitative determination

of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissue. *Anal Biochem* 1969; **27**: 502–22.
11. Adams JD Jr, Lauterburg BH, Mitchell JR. Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. *J Pharmacol Exp Ther* 1983; **227**: 749–54.
12. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* 1978; **86**: 271–8.
13. Weisel RD, Mickle DAG, Finkle CD, et al. Myocardial free-radical injury after cardioplegia. *Circulation* 1989; **80** (suppl III): 14–8.
14. Werns SW, Fantone JG, Ventura A, Lucchesi BR. Myocardial glutathione depletion impairs recovery of isolated blood-perfused hearts after global ischemia. *J Mol Cell Cardiol* 1992; **24**: 1215–20.
15. Lesnefsky EJ, Repine JE, Horowitz LD. Oxidation and release of glutathione from myocardium during reperfusion. *Free Radical Biol Med* 1989; **7**: 31–5.
16. Gates RN, Laks H, Drinkwater DC, et al. Gross and microvascular distribution of retrograde cardioplegia in explanted human hearts. *Ann Thorac Surg* 1993; **56**: 410–7.
17. Aronnson S, Lee BK, Zaroff JG, et al. Myocardial distribution of cardioplegic solution after retrograde delivery in patients undergoing cardiac surgical procedure. *J Thorac Cardiovasc Surg* 1993; **105**: 214–21.
18. Yau TM, Ikonomidis JS, Weisel RD, et al. Ventricular function after normothermic versus hypothermic cardioplegia. *J Thorac Cardiovasc Surg* 1993; **105**: 833–44.
19. Meister A, Anderson ME. Glutathione. *Annu Rev Biochem* 1983; **52**: 711–60.
20. Winterbourn C, Stern A. Human red cells scavenge extracellular hydrogenperoxide and inhibit formation of hypochlorous acid and hydroxyl radical. *J Clin Invest* 1987; **80**: 1486–91.
21. Brown JM, Grosso MJ, Terada LS, et al. Erythrocytes decrease myocardial hydrogen peroxide levels and reperfusion injury. *Am J Physiol* 1989; **256**: H584–8.
22. Magovern JR GE, Flaherty JT, Gott VL, Bulkley BH, Gradner TJ. Failure of blood cardioplegia to protect myocardium at low temperature. *Circulation* 1982; **60** (suppl I): 60–7.
23. Kukreja RC, Weaver AB, Hess ML. Stimulated human neutrophils damage cardiac sarcoplasmic reticulum function by generation of oxidants. *Biochim Biophys Acta* 1989; **990**: 198–205.
24. Liu X, Engelman RM, Wei Z, et al. Postischemic deterioration of sarcoplasmic reticulum: warm versus cold cardioplegia. *Ann Thorac Surg* 1993; **56**: 1154–9.