Warm Reperfusion and Myocardial Protection

Sidney Chocron, MD, PhD, Kifah Alwan, MD, Yusheng Yan, MD, Gérard Toubin, MD, Djamel Kaili, MD, Thierry Anguenot, MD, Leonardo Latini, MD, François Clement, MD, Jean-François Viel, MD, PhD, and Joseph-Philippe Etievent, MD

Departments of Thoracic and Cardiovascular Surgery, Pharmacology, Cardiology, and Public Health, Saint-Jacques Hospital, Besancon, France

Background. **The aim of this study was to determine whether warm reperfusion improves myocardial protection with cardiac troponin I as the criteria for evaluating the adequacy of myocardial protection.**

Methods. **One hundred five patients undergoing first-time elective coronary bypass surgery were randomized to one of three cardioplegic strategies of either (1) cold crystalloid cardioplegia followed by warm reperfusion, (2) cold blood cardioplegia followed by warm reperfusion, or (3) cold blood cardioplegia with no reperfusion.**

Results. **The total amount of cardiac troponin I released tended to be higher in the cold blood cardioplegia with no reperfusion group (3.9 ± 5.7 μg) than in the cold blood cardioplegia followed by warm reperfusion group (2.8 ± 2.7 μg) or the cold crystalloid cardioplegia followed by warm reperfusion group (2.8 ± 2.2 μg), but not significantly so. Cardiac troponin I concentration did not differ for any sample in any of the three groups.**

Conclusions. **Our study showed that the addition of warm reperfusion to cold blood cardioplegia offers no advantage in a low-risk patient group.**

(Ann Thorac Surg 1998;66:2003–7)

In a previous prospective randomized study [1] involving 70 patients with a preserved left ventricular function undergoing an elective first cardiac operation we compared crystalloid cardioplegia to cold blood cardioplegia followed by warm reperfusion using cardiac troponin I (CTnI) as the criteria for evaluating the adequacy of myocardial protection. The results clearly showed better myocardial protection to be provided by cold blood cardioplegia followed by warm reperfusion. As blood cardioplegia and warm reperfusion are usually used together, we do not know to what extent each element contributes to improved myocardial protection. To answer this question, we designed a study that included three types of myocardial protection: (1) crystalloid cardioplegia with warm reperfusion, (2) cold blood cardioplegia with warm reperfusion, and (3) cold blood cardioplegia with no warm reperfusion. The aim of our present prospective randomized study was to determine the extent to which cold blood cardioplegia and warm reperfusion have a bearing on the improvement of myocardial protection, with CTnI as the criteria for evaluating the adequacy of myocardial protection.

Patients and Methods

Patient Selection

One hundred five consecutive patients (90 men and 15 women; mean age, 65 ± 8 years) scheduled for first elective coronary artery bypass grafting from December 1996 to June 1997 agreed to participate in a prospective randomized trial comparing cold crystalloid cardioplegia followed by warm reperfusion (CWR), cold blood cardioplegia followed by warm reperfusion (BWR), and cold blood cardioplegia with no reperfusion (BC). The group into which a patient was randomized was known by the team just before incision. Not included in this study were patients requiring only one distal anastomosis, patients with an ejection fraction below 0.30, patients undergoing reoperation, and patients suffering from concomitant heart valve disease or unstable angina. Coronary artery stenoses causing a loss of 70% or more of the cross-sectional area were considered to be significant. For the left main coronary artery, a loss of 50% was considered significant.

Operative Technique

Cannulation for cardiopulmonary bypass was done in the usual fashion with a single-stage venous cannulation technique and moderate hypothermia (30°C). The left ventricle was vented by a catheter introduced through the right superior pulmonary vein. The route of delivery was exclusively antegrade in all groups. Cardioplegia was injected into the aortic root immediately after aortic cross-clamping and until cardiac arrest was achieved with a minimal amount of 700 mL. A dose of 150 mL was reinjected into the aortic root after each distal anastomosis, with the exception of the last one. No additional doses of cardioplegia were given through the vein grafts after completion of the distal anastomosis in either group. Myocardial protection was completed by additional topical cooling.

Accepted for publication June 5, 1998.

Address reprint requests to Dr Chocron, Department of Thoracic and Cardiovascular Surgery, Hopital Saint-Jacques, 25030 Besancon, France (e-mail: chocron@usa.net).

Published by Elsevier Science Inc

0003-4975/98/$19.00
PII S0003-4975(98)00909-6

Table 1. Cardioplegic Solutions

Variables	Crystalloid Solution Composition	Blood Cardioplegia Additive Composition	Approximate Blood Cardioplegia Final Concentration
Sodium (mmol/L)	147	147	140
Potassium (mmol/L)	20	70	20
Magnesium (mmol/L)	16	16	6
Calcium (mmol/L)	2	2	2
Chloride (mmol/L)	203	203	150
Bicarbonate (mmol/L)	0	0	16
Hematocrit (%)	0	0	20

The blood cardioplegia is a mixture (3:1 dilution) of the oxygenated blood of the patient and a hyperkalemic crystalloid concentration.

Cardioplegia Groups

The composition of the crystalloid cardioplegia is detailed in Table 1. Cardioplegia was administered with the Dideco D514 delivery set (Dideco Inc, Mirandola, Italy), which mixes and cools oxygenated blood with a hyperkalemic crystalloid concentration in a 3:1 dilution to achieve a final potassium concentration similar to that of the crystalloid solution used (Table 1). The temperature of cardioplegia ranged from 6° to 8°C in all groups. Warm reperfusion was started immediately after the last distal anastomosis, and was performed with a constant flow rate of 200 mL/min. Warm reperfusion was carried out in two steps and took 6 minutes: (1) during the first 2 minutes, the blood hyperkalemic mixture defined above was injected and progressively rewarmed to 20°C, and (2) during the last 4 minutes, the infusate used was exclusively composed of oxygenated blood, which increased to 35°C at the end of the reperfusion.

Measurements of Cardiac Marker Proteins

Serial venous blood samples were drawn just before cardiopulmonary bypass and after aortic unclamping at 6, 9, 12, and 24 hours and daily thereafter for 5 days. Cardiac troponin I concentrations were measured by a specific immunoenzymometric assay developed by ERIA Diagnostics Pasteur (Marne-la-Coquette, France). Each standard CTnI or test sample was incubated with monoclonal antibody 8E1 for 15 minutes. After washing, enzyme activity was measured following the addition of a substrate (tetramethylbenzydine). The reaction was stopped by adding H_2SO_4 and the absorbance was read at 450 nm on the status spectrophotometer. Creatine kinase isoenzyme MB was measured at hour 6.

Electrocardiogram

A 12-lead electrocardiogram was recorded preoperatively at 2 hours and then daily postoperatively. The electrocardiographic diagnosis criteria for perioperative myocardial infarction (PMI) were new Q-waves more than 0.04 ms and a reduction in R-waves more than 25% in at least two leads. The CTnI diagnosis criteria for PMI were CTnI peak concentrations more than 3.7 μg/L and CTnI concentration more than 3.1 μg/L at 12 hours or more than 2.5 μg/L at 24 hours as determined by Mair and colleagues [2]. We determined the number of patients having acquired conduction defects and the type of defect for each group.

Statistical Analysis

Sample sizes were calculated for a two-sided significance level $\alpha = 0.05$ and power $1\text{-}\beta = 0.8$ to detect a difference of 0.5 μg/L in CTnI concentration between groups. The standard deviation of measurements of CTnI was based on a previous study [3]. The number of patients required in each group was twenty-nine.

Statistical analysis was performed with BMDP statistical software (BMDP Corp, Los Angeles, CA). One-way analysis of covariance with repeated measures (BMDP 5V) was performed to test the effect of the type of cardioplegia and time on CTnI concentration. Two-way analysis of covariance with repeated measures was performed to test the effect of the type of cardioplegia and cross-clamping time, as well as the cardiopulmonary bypass time and the number of distal anastomosis on CTnI concentration.

Categoric data and quantitative variables were compared by the χ^2 test and the one-way analysis of variance, respectively. A p value less than 0.05 was considered statistically significant.

Results

Preoperative Data

No patient suffered from aortic incompetence. Preoperative and operative data are shown in Table 2. The preoperative ejection fraction was equivalent in all groups. The repartition of coronary angiographic data did not differ from one group to the other (Table 3).

Operative Data

At least one mammary artery was used in all patients. The number of distal anastomoses, cross-clamping time, and cardiopulmonary bypass time were significantly lower in the BC group. Sequential grafts were performed in 9 patients in the CWR group, 6 patients in the BWR group, and 3 patients in the BC group (p = not significant [NS]). The average amount of crystalloid solution injected was 1,250 ± 230 mL in the CWR group, 420 ± 100 mL in the BWR group, and 260 ± 70 mL in the BC group ($p < 0.01$). Mean core temperature immediately preceding aortic unclamping was the same in all groups (34.4°C). Spontaneous defibrillation occurred in 5 patients in the CWR group, 8 in the BWR group, and 2 in the BC group (p = NS). The difference between preoperative hematocrit and hematocrit drawn immediately preceding aortic unclamping (with no blood transfusion in either group) was higher in the CWR than in the other two groups (12.5% ± 2.8% in the CWR group, 10.5% ± 2.9% in the BWR group, 10.9% ± 3.0% in the BC group, $p < 0.05$).

Table 2. Patient Profile by Group

Variable	CWR Group (n = 35)	BWR Group (n = 35)	BC Group (n = 35)	*p* Value
Mean age (y)	63 ± 9	66 ± 9	66 ± 7	NS
Ejection fraction	0.56 ± 0.1	0.56 ± 0.1	0.55 ± 0.1	NS
Body surface area (m^2)	1.85 ± 0.20	1.91 ± 0.18	1.86 ± 0.18	NS
Anterior preoperative MI (n)	5	5	9	NS
Inferior preoperative MI (n)	14	14	11	NS
Distal anastomoses	98	90	83	<0.05
Distal anastomoses per patient (n)	2.8 ± 0.7	2.6 ± 0.7	2.4 ± 0.5	<0.05
LIMA used (n)	7	15	10	NS
LIMA+RIMA used (n)	28	20	25	NS
Saphenous vein grafts (n)	26	29	20	NS
Sequential grafts (n)	9	6	3	NS
Cross clamp time (min)	40 ± 12	37 ± 10	26 ± 7	<0.01
Pump time (min)	65 ± 19	65 ± 17	50 ± 16	<0.01
Amount of crystalloid cardioplegia (mL)	1250 ± 230	420 ± 100	260 ± 70	<0.01
Postoperative peak CK-MB (IU/L)	28 ± 13	26 ± 13	38 ± 46	NS
Total amount of CTnl (μg)	2.8 ± 2.2	2.8 ± 2.7	3.9 ± 5.7	NS
30-d mortality	0	0	1	NS

CWR = crystalloid cardioplegia with warm reperfusion; BWR = cold blood cardioplegia with warm reperfusion; BC = cold blood cardioplegia with no warm reperfusion; MI = myocardial infarction; LIMA = left internal mammary artery; RIMA = right internal mammary artery; CTnI = cardiac troponin I; CK-MB = creatine kinase-isoenzyme MB; NS = not significant. Where applicable, values are expressed as mean ± standard deviation.

Postoperative Data

One patient in the BC group died at day 10 from PMI. There were no cases of acquired left bundle branch block in the CWR group, 1 in the BWR group, and 3 in the BC group (p = NS). Six patients in the CWR group, 4 in the BWR group, and 3 in the BC group had an acquired right bundle branch block (p = NS). Acquired atrial fibrillation during the first 10 postoperative days occurred in 15 patients in the CWR group, 12 patients in the BWR group, and 14 in the BC group (p = NS).

One patient in the BWR group and 2 in the BC group had electrocardiographic evidence of PMI. One patient in the CWR group, 1 in the BWR group, and 3 in the BC group had CTnI evidence of PMI (p = NS). Conversely, all but 1 patient with electrocardiographic evidence of PMI had CTnI evidence of PMI. Twenty-two patients in the CWR group, 23 in the BWR group, and 23 in the BC group required no inotropic support (p = NS). Eight patients from the CWR group, 11 from the BWR group, and 6 from the BC group (p = NS) received either dopamine hydrochloride (3 to 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or dobutamine (3 to 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Five patients from the CWR group, 1 from the BWR group, and 6 from the BC group (p = NS) received epinephrine (0.2 to 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The total amount of CTnI released was higher in patients requiring inotropic support than in patients not requiring inotropic support (4.5 ± 5.0 μg versus 2.4 ± 2.3 μg, $p < 0.05$).

Table 3. Angiographic Data

Variable	CWR	BWR	BC	*p* Value
LMCA stenosis ≥50%				NS
No	24	28	23	
Yes	11	7	12	
LMCA stenosis ≥50%				NS
With normal RCA	5	1	4	
With diseased RCA	6	6	8	
No. of diseased vessels[a]				NS
One	0	1	0	excluded from analysis
Two	6	11	9	
Three	18	16	14	

LMCA = left main coronary artery; RCA = right coronary artery; CWR = crystalloid cardioplegia with warm reperfusion; BWR = blood cardioplegia with warm reperfusion; BC = blood cardioplegia with no warm reperfusion; NS = not significant.

[a] Stenosis of LMCA excluded.

Cardiac Troponin I Features

Figure 1 shows the time course of CTnI concentration according to the type of cardioplegia. None of the three curves differs significantly from the others. The total amount of CTnI released tended to be higher in the BC group (3.9 ± 5.7 μg) than in the BWR group (2.8 ± 2.7 μg) or the CWR group (2.8 ± 2.2 μg) but not significantly so. The CTnI concentration did not differ for any sample in any of the three groups. As the randomization was unbalanced due to the number of distal anastomoses, cross-clamping time, and cardiopulmonary bypass time, we performed a two-way analysis of covariance with repeated measures to take these factors into account. It

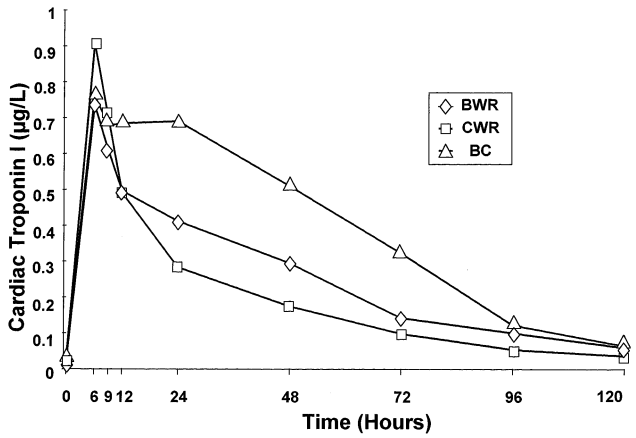

Fig 1. Time course of cardiac troponin I concentration according to type of cardioplegia. None of the three curves differ significantly from the others (p = not significant). With regard to the cardiac troponin I concentration of each sample, there was no significant difference between groups whatever the sample.

showed that these factors did not influence the results. Creatine kinase isoenzyme MB concentration at hour 6 was equivalent in all groups.

Comment

Cardiac troponin I is a specific marker of myocardial damage. This specificity is particularly beneficial for patients undergoing cardiac operation because the value of measurements of serum creatinine kinase and lactate dehydrogenase is limited by enzyme release from non-cardiac tissues [4]. The CTnI increases in all patients after cardiac operation [3]. This fact reflects the inevitable myocardial damage caused by cardioplegic arrest [2]. Mair and colleagues [2], in a study concerning patients undergoing coronary artery bypass grafting, concluded that a wide range of myocardial damage even in non-PMI is common and not always indicated by creatine kinase isoenzyme MB mass or activity. The CTnI measurements can detect these small differences in myocardial tissue damage. We have already performed studies showing (1) the sensitivity of CTnI to myocardial ischemia [3, 5], and (2) the interest of using CTnI to compare different methods of myocardial protection in cardiac operation [1, 6]. The interest of such comparisons is highlighted by the numerous methods of myocardial protection used [7] that demonstrate that there is no clear evidence to prefer one method over the others. Our last study [1] compared crystalloid cardioplegia with cold blood cardioplegia followed by warm reperfusion. This study concluded clearly that cold blood cardioplegia followed by warm reperfusion leads to better myocardial protection than crystalloid cardioplegia. The question remained whether the improvement in myocardial protection was attributable to cold blood, warm reperfusion, or to both. The aim of our present prospective randomized study was to determine the extent to which cold blood cardioplegia and warm reperfusion contribute to the improvement of myocardial protection using CTnI as the criteria for evaluating the adequacy of myocardial protection.

To answer this question, we designed a study including three types of myocardial protection. The results indicated no difference in myocardial protection between any of the three groups. The fact that the BWR group and the BC group were equivalent tends to demonstrate that the addition of warm reperfusion does not improve the myocardial protection provided by cold blood cardioplegia in a group of patients undergoing an elective first cardiac operation, and having a preserved left ventricular function. In our previous study [1] cold blood cardioplegia with warm reperfusion was clearly better than crystalloid cardioplegia with no warm reperfusion. In our present study, crystalloid cardioplegia with warm reperfusion is equivalent to cold blood cardioplegia with warm reperfusion. Comparison of the results of these two studies tends to show that the addition of warm reperfusion to crystalloid cardioplegia makes it as effective as cold blood cardioplegia with warm reperfusion.

It is somewhat disturbing that the improvement procured by warm reperfusion depends on the type of cardioplegia used.

Antegrade cold crystalloid cardioplegia is the simplest method of myocardial protection method to implement. The quiet bloodless field and flaccid heart provide optimal conditions for the cardiac surgeon. Cardioplegic crystalloid solutions preserve ventricular function, prevent depletion of high energy substrates, and maintain ultrastructural integrity [8, 9]. Julia and colleagues [10], in an experimental study, showed that crystalloid cardioplegia was deleterious because it is devoid of free radical scavengers.

Therefore, it is understandable that warm reperfusion minimizes reperfusion damage by washing out the substances produced by the anaerobic metabolism and by bringing free radical scavengers to a heart at rest. The comparison of the results of our current study to those of the previous one [1] suggests that there is a benefit of warm reperfusion to crystalloid cardioplegia. This should incite users of crystalloid cardioplegia to complete it by warm reperfusion.

The advantages of blood cardioplegia [11] include (1) oxygen delivery [12], (2) the buffering capacity of blood, (3) capillary flow distribution [11], (4) prevention of free radical generation [10], (5) maintenance of oncotic pressure [13], and (6) restriction of hemodilution. Most of the above-stated advantages of using cold blood are minimized by the low myocardial temperatures commonly achieved during clinical practice. Julia and colleagues [10] attributed the advantage of cold blood to the abundant quantity of free radical scavengers (catalase and reduced glutathione) in erythrocytes, which is a feature of blood cardioplegic solutions that "is sometimes overlooked in favor of their buffering capacity, oxygen-carrying capacity, and rheologic properties"[10].

Regarding warm reperfusion, in a prospective randomized trial including 20 patients undergoing elective coronary artery bypass grafting, Teoh and associates [14] compared cold blood cardioplegia (11 patients) to cold

blood cardioplegia followed by a "hot shot" (9 patients). They showed that with the hot shot, myocardial metabolic recovery was improved, high-energy phosphates were better preserved, metabolic response to stress was normal, and diastolic function was preserved. Reperfusion damage is thought to be caused in part by oxygen free radicals produced during the early phases of reoxygenation [15]. For Teoh and colleagues [14], the hot shot improves cold blood cardioplegia protection by washing out the products of anaerobic metabolism.

For Julia and colleagues [10] warm reperfusion limits damage because it contains endogenous free radical scavengers in red blood cells. Conversely to the study by Teoh and colleagues, our study showed no improvement when cold blood was completed by warm reperfusion. A possible explanation for the difference in the results is that the cross-clamping time in the study by Teoh and associates [14] was more than twice as long as ours (75 ± 19 minutes versus 26 ± 7 minutes in the BC group and 66 ± 10 minutes versus 37 ± 10 minutes in the BWR group) noting that warm reperfusion lasted 6 minutes in our study and 1 minute in the one by Teoh. Therefore, although the release of free radicals remains low (because of short clamping times or efficacy of cold blood cardioplegia), their washout or scavenge might not lead to a significant improvement in a low risk patient group undergoing fairly limited ischemic stress. The extra protection that is probably afforded by warm reperfusion is likely to be greatest in a higher risk patient group or when aortic cross-clamping is longer.

With regard to the preoperative and operative data (Table 1), randomization gave two equivalent groups for most of the variables studied. The time needed to perform one distal anastomosis was equivalent in the three groups. The number of bypasses and consequently the aortic cross-clamping time and the cardiopulmonary bypass time were significantly lower in the BC group. The inclusion of these covariates in the analysis did not influence the results.

Some of the results we obtained are the same as those stated in our previous study [1]: (1) the need for inotropic treatment was related to myocardial damage. CTnI was elevated in patients requiring inotropic support compared with patients requiring no inotropic support. (2) Restriction of hemodilution (a classic and logical advantage of BC) is again present but always with a difference (1.6%) of no clinical importance, and (3) the number of PMIs differs depending on the criteria chosen.

The higher rate of PMI detected by CTnI, as compared to that detected by the electrocardiogram, shows that measurement of CTnI allows the diagnosis of small perioperative necrotic myocardial areas that do not necessarily fulfill routine criteria for PMI. Mair and colleagues [2] showed that CTnI was able to detect non-Q-wave PMI.

In conclusion, our study showed that the addition of warm reperfusion to cold blood cardioplegia offers no advantage in low risk patients. This conclusion cannot be extended to high risk patients (eg, redo operations, combined surgery, ejection fraction below 0.30, unstable angina, or when aortic cross-clamping times are long). This is all the more so as the total amount of release of CTnI tends to be higher in the group with no warm reperfusion. The comparison of the results of our current study to those of the previous one suggests that much is to be gained by adding warm reperfusion to crystalloid cardioplegia. This should incite users of crystalloid cardioplegia to complete it by warm reperfusion.

We are grateful to Nancy Richardson-Peuteuil for her editorial assistance.

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