# INTERMITTENT ANTEGRADE WARM CARDIOPLEGIA REDUCES OXIDATIVE STRESS AND IMPROVES METABOLISM OF THE ISCHEMIC-REPERFUSED HUMAN MYOCARDIUM

**The aim of this study was to compare the effect of intermittent antegrade warm blood cardioplegia and intermittent antegrade cold blood cardioplegia on myocardial metabolism and free radical generation of the ischemic-reperfused human myocardium. Thirty patients undergoing mitral valve procedures were randomly allocated to two groups: group 1 (15 patients) received warm blood cardioplegia and group 2 (15 patients), cold blood cardioplegia. Myocardial metabolism was assessed before aortic clamping, 1 minute after crossclamp removal, and after 20 minutes of reperfusion, by collecting blood simultaneously from the radial artery and coronary sinus. All samples were analyzed for lactate, creatine kinase, reduced and oxidized glutathione, ascorbic acid, fluorescent products of lipid peroxidation, and leukocyte activation (elastase). In all patients, early reperfusion was associated with significant coronary sinus lactate release. In group 2, but not in group 1, significant coronary sinus release of reduced and oxidized glutathione, fluorescent products of lipid peroxidation, and creatine kinase was also found; moreover, arterial-coronary sinus difference of ascorbic acid content was increased only in group 2, suggesting a transmyocardial consumption of this antioxidant vitamin. After 20 minutes of reperfusion, coronary sinus lactate release was no longer present in group 1, whereas significant production was still evident in group 2. In this group, significant coronary sinus release of fluorescent products of lipoperoxidation and reduced and oxidized glutathione was also observed at this time. No significant release of elastase from the coronary sinus was noted in the two groups throughout the study. The left ventricular stroke work index measured at the end of the study indicated a better functional recovery in group 1 than in group 2. In conclusion, intermittent antegrade warm blood cardioplegia protects the myocardium from ischemia-reperfusion injury better than intermittent antegrade cold blood cardioplegia; this phenomenon may be partly due to the decreased tissue oxidant burden mediated by intermittent warm blood cardioplegia. (J THORAC CARDIOVASC SURG 1995;109:787-95)**

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Termination of ischemia by the resumption of coronary perfusion, although necessary for myocardial recovery, can result in a paradoxic extension of ischemic damage, the so-called ischemia-reperfusion injury.[1] A large body of evidence indicates that this injury is mediated by oxygen-derived free radicals,[2, 3] which are maximally produced at the onset of myocardial reperfusion and induce biomembrane peroxidation.[4, 5] Free radical generation and oxidative stress are evident in the ischemic reperfused human myocardium[6-9]; accordingly, antioxidant administration has been reported to result in a dramatic improvement in postoperative outcome.[10]

Hypothermia is considered an important ingredient during cardioplegic arrest because it slows myocardial metabolism and reduces ischemia-reperfusion-induced free radical generation.[10-12] Recent studies, however, have demonstrated myocardial depletion of alfa-tochopherol and enhanced coro-

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**Table I.** *Delivery protocol of warm intermittent antegrade blood cardioplegia*

| Dose | Flow rates: Blood (ml/min) | Flow rates: $K^+$ (ml/hr) | Duration (min) | $[K^+]$* (mEq/L) |
|---|---|---|---|---|
| 1 | 300 | Push 2 ml, then 150 | 2 | 18-20 |
| | *Following doses at 15 min intervals* | | | |
| 2 | 200 | 120 | 2 | 20 |
| 3 | 200 | 90 | 2 | 15 |
| 4 | 200 | 60 | 3 | 10 |
| 5 | 200 | 60 | 4 | 10 |
| 6 | 200 | 60 | 5 | 10 |

*The values are referred to the blood potassium concentration $[K^+]$ obtained by infusion of potassium at the corresponding flow rates, and they are independent on the intrinsic blood potassium values of each patient.

nary sinus release of lipid peroxides despite cold blood cardioplegia.[13] These results suggest that this cardioprotective approach may not afford a "full antioxidant" protection for the ischemic-reperfused human myocardium.

Normothermic cardioplegia has been shown to yield good metabolic and clinical results.[14-18] In this regard, intermittent antegrade warm blood cardioplegia has recently been proposed and standardized in a clinical setting as routine myocardial protective technique during cardiopulmonary bypass (CPB).[19]

Because normothermic blood cardioplegia results in less severe myocardial ischemia-reperfusion injury than cold blood cardioplegia[20] and free radicals and oxidative stress are involved in the heart damage associated with ischemia and reperfusion,[1-10] we hypothesized that normothermic blood cardioplegia might decrease myocardial oxidant load during ischemia and reperfusion.

This study was designed to compare the effect of intermittent antegrade warm blood cardioplegia and intermittent antegrade cold blood cardioplegia on myocardial metabolism and free radical generation at reperfusion after cardioplegic arrest. The coronary sinus levels of fluorescent lipoperoxidation products, reduced and oxidized glutathione (GSH and GSSG), and ascorbic acid were used as recognized indexes of myocardial oxidative stress.[3, 9, 20-22]

## Patients and methods

Thirty patients scheduled for elective mitral valve procedures by one surgeon (A.M.C.) agreed to participate in a study of alternative cardioplegic techniques. All patients signed a consent form approved by the Human Ethical Committee of the University of Chieti.

**Surgical technique.** CPB was established via two separate venous cannulas, with direct cannulation of the superior vena cava. During CPB, hemoglobin value was maintained at 6 to 8 gm/dl and pump flow ranged from 2.4 to 2.8 L/min per square meter. Mean arterial pressure was kept at 50 to 60 mm Hg, with the administration of sodium nitroprusside or metaraminol if necessary. The systemic temperature was maintained at 37° C in the normothermic group, whereas patients receiving cold blood cardioplegia were cooled systemically to 27° C.

**Cardioplegic groups and protocol for delivery of cardioplegic solution.** Patients included in the study were randomly allocated to one of two groups: group 1 (15 patients) received intermittent antegrade warm blood cardioplegia and group 2 (15 patients) received intermittent antegrade cold blood cardioplegia. The cardioplegic solutions were prepared by mixing blood with potassium. The composition of the cardioplegic solutions was as follows (in millimoles per liter): NaCl, 115; KCl, 20; $MgCl_2$, 15.5; $CaCl_2$, 1.3; $NaHCO_3$, 12; and tromethamine, 4.

In group 1 (warm blood cardioplegia), blood was taken directly from the oxygenator by a ¼-inch tubing and was infused at 37° C into the aortic root by means of a roller pump. A syringe pump was connected to the ¼-inch tubing to deliver potassium solution. The syringe contained 50 ml of a solution with 2 mEq of potassium per milliliter. The first dose lasted 2 minutes at a flow rate of 300 ml/min (600 ml overall). The syringe pump rapidly pushed 2 ml of the potassium solution in about 20 seconds, and then the flow rate was adjusted at 150 ml/min for a final concentration of about 20 mEq/L. After each distal anastomosis or after 15 minutes of normothermic ischemia, a second dose was repeated, at a flow rate of 200 ml/min (blood) and 120 ml/hr (potassium) for 2 minutes. Flow rates of blood and potassium delivery and duration of each dose are listed in Table I.

In group 2 (cold blood cardioplegia), blood was collected in a cardiotomy reservoir (DIDECO 715, Dideco S.P.A., Mirandola, Italy) with a separate heat exchanger and a recirculating circuit, to maintain a temperature of about 8° to 10° C.

The first dose was delivered at a flow rate of 300 ml/min during 3 minutes (900 ml overall); the longer infusion time of the first dose in group 2 (cold blood) as compared with group 1 (warm blood) allowed the heart to reach the appropriate myocardial temperature (i.e., 8° to 10° C). Further doses were similar to those used in group 1, as reported in Table I. In particular, the last dose was always comparable for volume but not for temperature (37° C versus 8° to 10° C) to intermittent antegrade warm blood cardioplegia. The final volume of cardioplegic solutions was similar for the two groups. In any case, reperfusion was obtained by removal of the aortic crossclamp in all patients.

The approach to the mitral valve was through the right atrium, the interatrial septum, and the roof of the left atrium. Before aortic crossclamping, the right atrium was opened and a soft catheter was introduced into the coronary sinus and fixed with a 5-0 Prolene suture (Ethicon, Inc., Somerville, N.J.).

Myocardial metabolism was assessed before aortic clamping, 1 minute after crossclamp removal, and after 20 minutes of reperfusion by collecting blood simultaneously from the radial artery and the coronary sinus. Blood for all the determinations, except for GSH and GSSG, was collected in brown tubes with heparin and immediately centrifuged at 4° C; plasma was separated and stored at −80° C in liquid nitrogen. All samples were analyzed for blood gases, pH, lactate, leukocyte activation (elastase), creatine kinase (CK), GSH, GSSG, ascorbic acid, and lipid peroxides (fluorescent products of lipoperoxidation).

**Measurement of GSH and GSSG.** GSH and GSSG were assessed as previously described.[22] Blood samples simultaneously drawn from arterial and coronary sinus catheters were immediately divided into separate tubes containing GSH and GSSG collection buffer. GSH collection buffer consisted of 0.5 ml of potassium phosphate buffer (0.1 mol/L), pH 7.4, 5,5′-dithiobis(2-nitrobenzoic acid) (3 mmol/L), and ethylenediaminetetraacetic acid (5 mmol/L). Supernatants for GSH were immediately frozen at −70° C for subsequent analysis.

GSSG collection buffer consisted of 0.3 ml of potassium phosphate buffer (0.1 mol/L), pH 6.5, with *N*-ethylmaleimide (10 mmol/L) and 5 ethylenediaminetetraacetic acid (5 mmol/L). *N*-ethylmaleimide reacts with reduced sulfhydryl groups (such as GSH) and prevents their detection in the assay of GSSG. Supernatants for GSSG analysis were then processed to remove excess *N*-ethylmaleimide, as previously described.[22] An adaptation of the assay devised by Tietze[23] and modified by Adams, Lauterburg, and Mitchell[24] was used to measure GSH and GSSG. Grossly hemolyzed samples occurred rarely and were discarded. The possibility of minor hemolysis was checked by procedures capable of detecting 0.2% free hemoglobin.[25] Experiments to assess efficiency of recovery were performed by adding known amounts of GSH (10 to 100 nmol/ml) and GSSG (0.5 to 5 nmol/ml) to plasma samples. Recoveries of GSH and GSSG were excellent. Results are expressed as nanomoles per deciliter.

**Ascorbic acid assay.** Ascorbic acid content in plasma was assessed as previously described.[26] In brief, 2 ml of trichloroacetic acid (0.615 mol/L) was added to 1 ml of plasma. After 5 minutes of preincubation at room temperature, the mixture was centrifuged at 2000 rpm for 10 minutes. Then, to 1 ml of this supernatant was added 2.5 ml of a solution containing 2,4,6-tripyridyl-*s*-triazine (0.24 mmol/L) and iron chloride (80 μmol/L) in sodium acetate buffer plus 20% methanol (vol/vol), pH 5.9. Absorbance values at 595 nm were then recorded on a double-beam Varian DMS 200 spectrophotometer (Varian Techtron Pty, Ltd., Melbourne, Australia) against an appropriate blank. Results were expressed as micromoles of vitamin C per liter.

**Fluorescent products of lipid peroxidation.** Lipid peroxidation was evaluated via assessment of fluorescent damage products of lipid peroxidation, which essentially reflect the interaction of peroxidation aldehydes with phospholipids.[27]

Total lipid was extracted from plasma as previously described.[27] The lipid-containing phase was removed and the samples were dried under a stream of nitrogen gas at room temperature.[28] The dried samples were resuspended in chloroform. To 1 ml of the chloroform layer 0.1 ml of methanol was added and the fluorescence values were estimated spectrofluorometrically at 360 and 430 nm excitation and emission,[27, 28] respectively, with a Kontron SFM25 spectrofluorometer (Kontron Instruments, Inc., Everett, Mass.) calibrated with quinine sulfate. The results were expressed as units of relative fluorescence per milliliter of plasma.

**Lactate assay.** Blood samples for lactate concentration were mixed with a measured volume of 6% perchloric acid. Lactate concentration was determined in the protein-free supernatant by an enzymatic method. The results were expressed in millimoles per liter.

**CK and elastase.** Serum CK was analyzed by a standard spectrophotometric method. Leukocyte activation was assessed by measuring elastase levels by an immunoactivation assay[16] (Merck-Clévenot, Chelles, France).

**Hemodynamic measurements.** Hemodynamic data were obtained by a radial arterial catheter and a thermistor-tipped pulmonary catheter (Swan-Ganz model 93A-831H-7.5F, Baxter Healthcare Corp., Edwards Div., Irvine, Calif.). Pulmonary capillary wedge pressure, radial artery pressure, and cardiac output were recorded. The cardiac index and left ventricular stroke work index were calculated.[29] The hemodynamic parameters were measured before the operation and 20 minutes after the end of CPB. Until this time, no patients received inotropic or vasoactive drugs.

**Statistical analysis.** Data are expressed as mean ± standard error of the mean, unless otherwise indicated; statistical significance was assumed at a probability level of less than 0.05. Differences in hemodynamic parameters within the groups were tested by paired Student's *t* test. An analysis of variance for repeated measures was used to test the effects of cardioplegia on biochemical parameters; when analysis of variance indicated a difference at the 5% level or less, Scheffé's test was used for individual group comparisons. Differences between the two groups were tested by unpaired Student's *t* test.

## Results

The clinical profiles are presented in Table II. The two groups were not significantly different regarding age, sex, and CPB time. The aortic crossclamping time was slightly longer in the warm blood than in the cold blood cardioplegia group. This difference was not statistically significant, although we cannot exclude that with a larger database a significant trend could have been obtained. However, the metabolic differences observed between the two groups are striking and not an expression of a mere trend. Cold cardioplegic solution was delivered during 12.6% ± 1.05% of the crossclamping period, and warm cardioplegic solution was infused during 9.5% ± 0.83% of the crossclamping interval ($p = 0.025$). This difference was essentially related to the longer infusion time of the first dose of cold cardioplegic solution; the shorter ischemic time in

**Table II.** *Summary of clinical data*

| | *Group 1* | *Group 2* | p *Value* |
|---|---|---|---|
| Patients *(n)* | 15 | 15 | NA |
| Age (yr) | 63 ± 12.8 | 69 ± 4.7 | NS |
| Sex (M/F) | 8/7 | 9/6 | NA |
| NYHA class (II/III/IV) | 8/6/1 | 8/5/2 | NA |
| CPB time (min) | 76.4 ± 9.6 | 83 ± 18.6 | NS |
| Aortic clamping time (min) | 56.5 ± 8.7 | 62.5 ± 16 | NS |

Data are shown as mean ± standard deviation of the mean; *group 1,* warm intermittent antegrade blood cardioplegia; *group 2,* cold intermittent antegrade blood cardioplegia; *NYHA,* New York Heart Association; *NA,* statistics not applicable; *NS,* not significant.

**Table III.** *Summary of hemodynamic data of 30 patients before and at the end of CPB**

| | *Before CPB* | *After CPB* | p *Value* |
|---|---|---|---|
| Group 1 | | | |
| HR (beats/min) | 76 ± 7 | 87 ± 7 | NS |
| CI (L/min per square meter) | 2.70 ± 0.27 | 2.96 ± 0.14 | NS |
| MAP (mm Hg) | 80 ± 8 | 75 ± 4 | NS |
| PCWP (mm Hg) | 11.4 ± 1.9 | 9.4 ± 1.4 | NS |
| LVSWI (gm · m/m$^2$) | 44.6 ± 3.6 | 40 ± 6.5 | NS |
| Group 2 | | | |
| HR (beats/min) | 74 ± 5 | 82 ± 4 | NS |
| CI (L/min per square meter) | 2.68 ± 0.21 | 2.35 ± 0.15 | NS |
| MAP (mm Hg) | 86 ± 5 | 89 ± 7 | NS |
| PCWP (mm Hg) | 10.5 ± 1.6 | 12.2 ± 1.8 | NS |
| LVSWI (gm · m/m$^2$) | 43.2 ± 2.3 | 34.4 ± 2.6 | 0.017 |

*Data are shown as means ± standard error of the mean; *group 1,* warm intermittent antegrade blood cardioplegia; *group 2,* cold intermittent antegrade blood cardioplegia; *HR,* heart rate; *CI,* cardiac index; *MAP,* mean arterial pressure; *PCWP,* pulmonary capillary wedge pressure; *LVSWI,* left ventricular stroke work index; *NS,* not significant.

the warm than in the cold blood group, although not statistically significant (Table II), could have contributed to the aforementioned difference.

Hemodynamic parameters for both groups before and after CPB are reported in Table III. No significant change in hemodynamic parameters was observed in group 1 (warm blood cardioplegia), whereas significantly lower left ventricular stroke work index (34.4 ± 2.6 versus 43.2 ± 2.3 gm × m/m$^2$, $p = 0.017$) was found in group 2 at 20 minutes after the end of CPB as compared with before crossclamping. Even though the measurement of left ventricular stroke work index appears to be load dependent,[15] no patients received inotropic or vasoactive drugs until the hemodynamic study; moreover, as shown in Table III, the levels of mean arterial pressure (expression of afterload) and of pulmonary capillary wedge pressure (which may be considered an expression of preload) were not significantly different between the two groups.

**Metabolic data**

*Changes in plasma glutathione levels.* In both groups arterial GSH levels remained stable during the reperfusion period. No significant changes in coronary sinus concentrations of GSH and GSSG were observed in group 1 throughout reperfusion. In contrast, a marked and significant coronary sinus release (Fig. 1) of both GSH and GSSG was found in group 2 (−161 ± 19 nmol/dl, $p < 0.0001$; −23.9 ± 3.3 nmol/dl, $p < 0.0001$, respectively). In this group, the arterial–coronary sinus differences for GSH and GSSG were still negative after 20 minutes of reperfusion (−75 ± 14 nmol/dl, $p < 0.0001$; −14.6 ± 1.7 nmol/dl, $p < 0.0001$, respectively).

*Fluorescent products of lipid peroxidation.* Fig. 2 shows the mean values of arterial–coronary sinus differences for fluorescent lipoperoxidation products. At 1 minute after crossclamp removal, coronary sinus concentrations of fluorescent lipoperoxidation products were significantly higher than the arterial levels in group 2 ($p < 0.0001$), whereas no significant difference was found in group 1. At 20 minutes of reperfusion, the concentrations were still significantly higher in the coronary sinus than in the arterial blood ($p < 0.0001$). On the contrary, no significant arterial–coronary sinus difference was found in group 1.

*Ascorbic acid levels.* Arterial levels of ascorbic acid did not change significantly throughout the study. In group 1, levels of ascorbic acid in the coronary sinus were not significantly different from arterial concentrations. Conversely, a significant increase in arterial–coronary sinus difference (Fig. 2) was found in group 2 after release of the crossclamp with respect to baseline (from 0.41 ± 0.3 μmol/L to 4.35 ± 0.5 μmol/L, $p < 0.0001$). In this group, ascorbic acid level was still significantly lower in the coronary sinus than in the artery at 20 minutes of reperfusion ($p < 0.0001$).

*Lactate, CK, and elastase plasma levels.* A significant release of lactate from the coronary sinus occurred in both groups 1 minute after unclamping (Fig. 3); this release was significantly higher in group 1 than in group 2 ($p < 0.05$). After 20 minutes of reperfusion, a significant myocardial lactate production was still present in group 2, but lactate release was completely reversed to extraction in group 1. No significant changes in CK arterial–coronary sinus

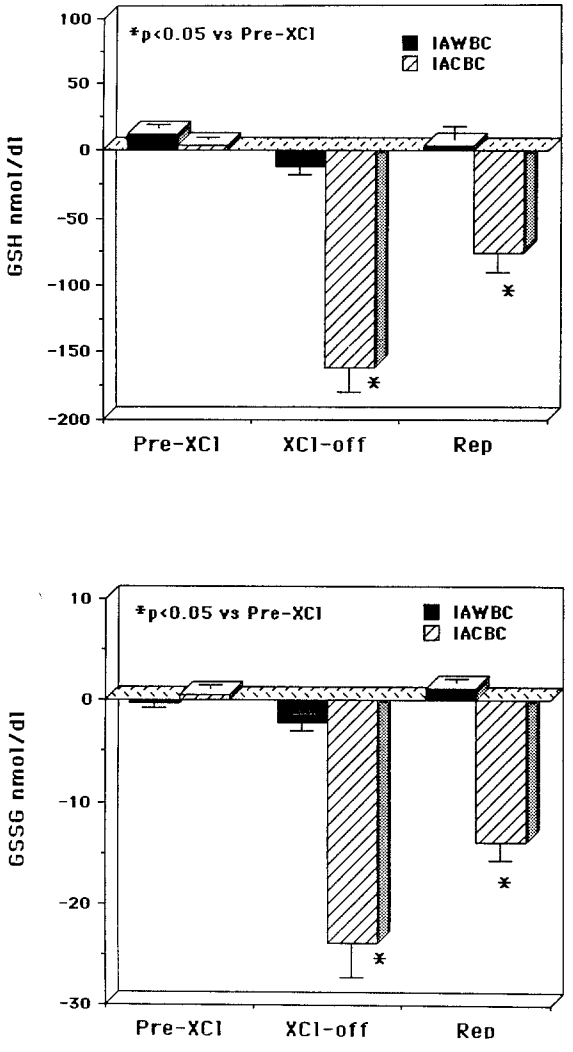


**Fig. 1.** Time course of changes in arterial–coronary sinus differences of plasma GSH and GSSG. Data are expressed as means ± standard error of the mean. *PreXCl*, Before aortic crossclamping; *XCl-off*, 1 minute after crossclamp removal; *Rep*, after 20 minutes of reperfusion; *IAWBC*, intermittent antegrade warm blood cardioplegia; *IACBC*, intermittent antegrade cold blood cardioplegia.

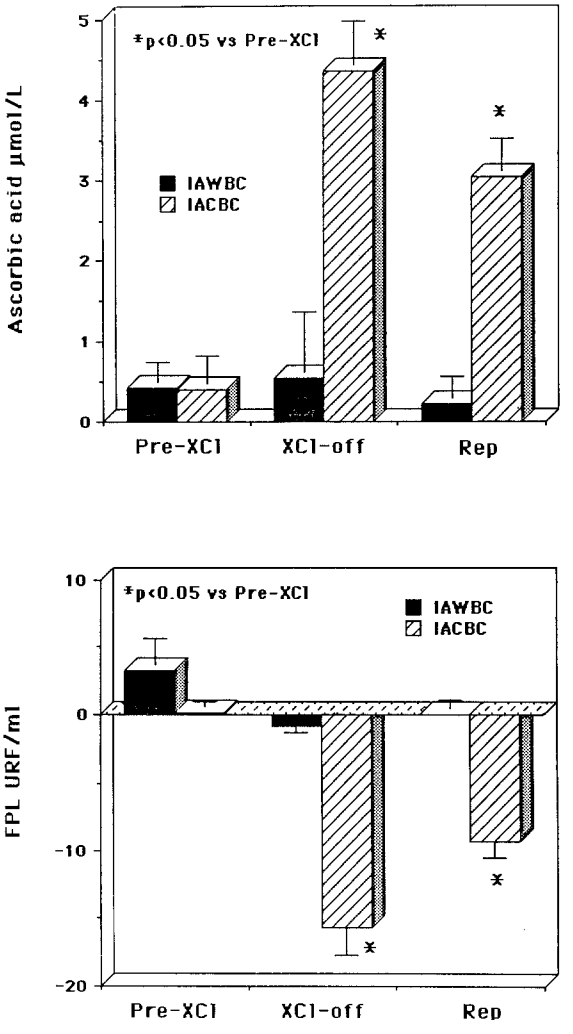


**Fig. 2.** Time course of changes in arterial–coronary sinus differences of plasma fluorescent products of lipid peroxidation *(FPL)* and ascorbic acid. Data are expressed as means ± standard error of the mean. *PreXCl*, Before aortic cross-clamping; *XCl-off*, 1 minute after crossclamp removal; *Rep*, after 20 minutes of reperfusion; *IAWBC*, intermittent antegrade warm blood cardioplegia; *IACBC*, intermittent antegrade cold blood cardioplegia.

differences were found throughout the study in group 1 (Fig. 3), whereas a significant CK release was observed in group 2 at 1 minute after unclamping; the release was markedly reduced but still significant at 20 minutes of reperfusion. As shown in Fig. 4, arterial elastase levels increased over time in both groups. This increase was significantly higher in group 2 than in group 1 throughout reperfusion. However, coronary sinus elastase values were not significantly different from arterial levels in either group throughout the study.

## Discussion

Our data suggest that oxidative stress is avoided in hearts protected by intermittent antegrade warm blood cardioplegia. In fact, in group 1 coronary sinus concentrations of GSH, GSSG, and fluorescent lipoperoxidation products never exceeded arterial levels (see Figs. 1 and 2) throughout the reperfusion period, a finding that seems to rule out the occurrence of myocardial oxidative stress. On the contrary, a significant coronary sinus release of GSH, GSSG, and fluorescent lipoperoxidation

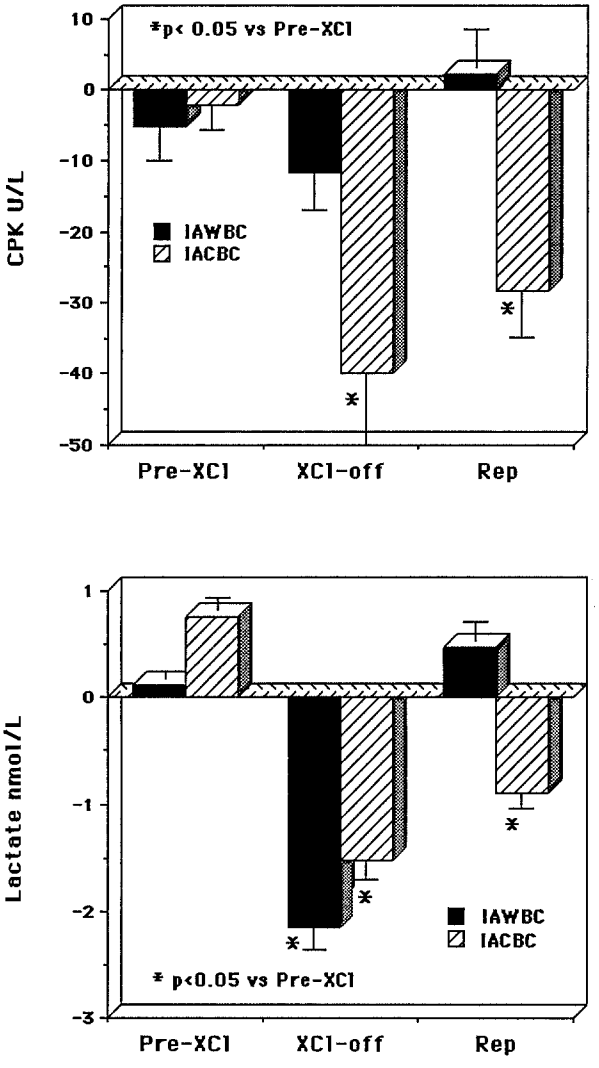


**Fig. 3.** Time course of changes in arterial–coronary sinus differences of plasma levels of creatine kinase *(CPK)* and lactate. Data are expressed as means ± standard error of the mean. *PreXCl,* Before aortic crossclamping; *XCl-off,* 1 minute after crossclamp removal; *Rep,* after 20 minutes of reperfusion; *IAWBC,* intermittent antegrade warm blood cardioplegia; *IACBC,* intermittent antegrade cold blood cardioplegia.

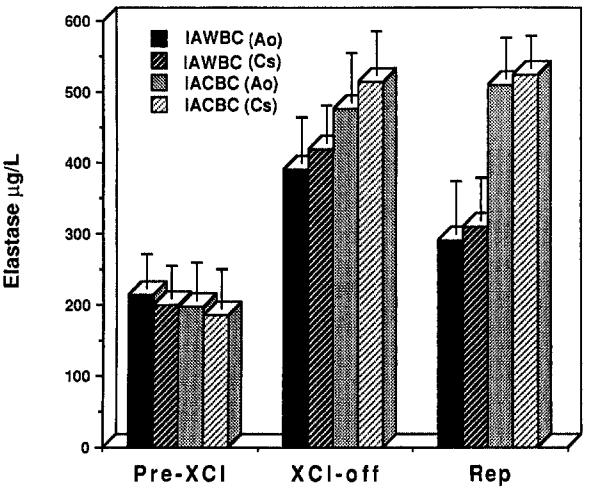


**Fig. 4.** Time course of changes in arterial and coronary sinus elastase concentration (leukocyte activation). Data are expressed as means ± standard error of the mean. *PreXCl,* Before aortic crossclamping; *XCl-off,* 1 minute after crossclamp removal; *Rep,* after 20 minutes of reperfusion; *Ao,* aorta; *Cs,* coronary sinus; *IAWBC,* intermittent antegrade warm blood cardioplegia; *IACBC,* intermittent antegrade cold blood cardioplegia.

products (see Figs. 1 and 2) was present in group 2 during reperfusion. These data are indicative of myocardial oxidative stress and myocyte biomembrane alterations.[3, 9, 20-22] However, some differences observed between the two groups may be partly related to a potentially lower coronary flow in the patients treated by cold blood cardioplegia (group 2), presumably as a result of cold-induced coronary constriction. The loss of cellular GSH represents an important rate-limiting factor for the reaction involved in cell detoxification from oxygen-reactive intermediates.[30, 31] Moreover, the increased myocardial production and release of GSSG, caused by cellular incapability to regenerate GSH from its oxidized form, is considered a sensitive and specific index of intracellular oxidative stress.[3, 9, 22, 30, 31] Thus in group 2 the readmission of normothermic fully oxygenated blood at reperfusion seems to favor the shifting of the oxidation-reduction state of the cells toward oxidation, resulting in free radical–mediated cellular damage.

Accordingly, in this group ascorbic acid content was always significantly lower in the coronary sinus than in the arterial blood during reperfusion, suggesting a transmyocardial consumption of this vitamin (see Fig. 2). In contrast, no significant difference between coronary sinus and arterial concentrations of this antioxidant was evidenced in group 1 throughout the study.

Ascorbic acid represents the outstanding antioxidant in the plasma environment,[32] whereas the antioxidant properties of vitamin E are essentially operative at biomembrane level.[33] Indeed, free radical–driven plasma peroxidation begins only when

vitamin C is depleted, even though vitamin E is still present at high concentrations.[34]

Reportedly, the most important sources of free radicals in the myocardial cell are represented by the mitochondrial electron transport system, the arachidonic pathway and activated neutrophil, or, in some species, xanthine oxidase.[1-3] However, xanthine oxidase activity is not present in homogenates of the whole human heart,[35] although the enzyme seems to be localized at the capillary endothelium level of human myocardium.[36] This finding could explain the beneficial effect of allopurinol in decreasing oxidative stress in human hearts, although both a metabolic action and direct free-radical scavenging properties of the drug and its metabolite, oxypurinol, may be operative.[37, 38] In our study leukocyte activation was assessed by the release of elastase, which is a protease stored in azurophilic granules of polymorphonuclear leukocytes.[39] According to previous studies,[40, 41] a significant increase in elastase arterial levels was found at reperfusion in both groups (Fig. 4). However, the absence of significant transmyocardial elastase production seems to rule out the possible role of an increased neutrophil activation within the myocardium. It has recently been reported that hypothermia may induce a significant mitochondrial dysfunction at the level of the electron transport chain.[42, 43] After release of the crossclamp, the reperfusion of the cold ischemic myocardium with almost normothermic fully oxygenated blood, in the presence of a depressed mitochondrial function, may result in an increased cellular oxidative burden.

The data also show that warm rather than cold cardioplegia better protects the heart from ischemia-reperfusion injury. Accordingly, the absence of a significant CK coronary sinus release in group 1 (see Fig. 3) during the reperfusion confirms the hypothesis of a more effective protection given by warm cardioplegia. In group 2, the reperfusion-induced release of CK and GSH reflects both increased cellular membrane permeability and damage. The finding that in group 1 coronary sinus lactate release was limited to the immediate postreperfusion period (see Fig. 3), whereas in group 2 lactate myocardial production persisted during the entire reperfusion period, is consistent with a more rapid recovery of aerobic metabolism in the hearts protected by warm cardioplegia. In these hearts the coronary sinus lactate release, at the time of aortic crossclamp release, largely reflects the washout of the metabolite accumulated during CPB rather than a continued anaerobic myocardial activity. Moreover, the higher lactate washout at reperfusion in group 1 than in group 2 could be an expression of a more elevated glycolytic activity and anaerobic production of adenosine triphosphate. In this regard, it is worth noting that glycolytic adenosine triphosphate is considered relevant for membrane function and integrity,[44, 45] so that this mechanism could contribute to the cardioprotective effect of intermittent antegrade warm blood cardioplegia. Furthermore, free radicals or GSSG have been shown to inhibit the oxidative metabolism of lactate.[46] Thus, prevention of oxidative stress by intermittent antegrade warm blood cardioplegia may, in turn, favor a more rapid recovery of myocardial aerobic metabolism at reperfusion.

Antioxidant repairing mechanisms, such as regeneration of GSH, are energy-dependent phenomena.[3, 30] It has recently been demonstrated that normothermia increases myocardial oxygen delivery and consumption during cardioplegia and reperfusion, favoring mitochondrial oxidative phosphorylation and adenosine triphosphate regeneration from adenosine diphosphate and monophosphate.[20, 47] In contrast, hypothermia seems to alter tissue oxygen delivery and reduce the cell's ability to produce adenosine triphosphate efficiently after a period of anaerobic arrest.[18, 20] Furthermore, it is widely known that the activities of many enzymes involved in crucial metabolic pathways such as glycolysis or free radical detoxification are inhibited by low tissue temperatures.[18, 42] Finally, the superiority of warm as compared with cold blood cardioplegia in protecting the myocardium during CPB is further supported by hemodynamic data showing a better functional recovery in group 1 than in group 2.

In conclusion, intermittent antegrade warm blood cardioplegia provides better metabolic and functional recovery for the ischemic-reperfused human myocardium than does intermittent antegrade cold blood cardioplegia. This effect may be partly related to a decreased myocardial oxidant burden.

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