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Comparison between warm blood and crystalloid cardioplegia during open heart surgery

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Abstract

Objective: This study was designed to compare the degree of myocardial protection afforded by warm blood and cold crystalloid cardioplegia in a group of patients undergoing elective coronary artery bypass surgery. *Methods:* Seventeen patients, were randomly assigned to Group A ($n=9$), who received crystalloid cardioplegic solution, and Group B who received warm blood cardioplegic solution ($n=8$). Before the aorta was clamped, and 10 min after reperfusion, blood samples from the coronary sinus were obtained to assay α-tocopherol, β-carotene, ubiquinol, and thiobarbituric acid reactive substances (TBARS). At the same intervals, biopsies from the left ventricle were obtained to determine ultrastructural alterations. *Results:* No significant changes were observed between preischemia and reperfusion values for both blood and crystalloid groups concerning α-tocopherol, β-carotene, and ubiquinol, and no differences between groups were detected. Values for TBARS in group A were 3.49 ± 0.3 and 5.27 ± 0.45 μM for presichemia and reperfusion samples, respectively ($P<0.01$). In group B values were 2.6 ± 0.3 and 3.54 ± 0.3 μM, respectively ($P=$NS). For electron microscopy studies, semiquantitative analysis showed a significant mitochondrial damage in reperfusion biopsies from group A (grades 0, 3 and 4). In group B, no significant changes were observed in mitochondrial damage between preischemia and repefusion biopsies (except for grade 0). *Conclusion:* These results indicate that blood cardioplegia affords better protection to the myocyte than crystalloid cardioplegia.

Keywords: Cardioplegia; Warm blood; Crystalloid; Heart surgery; Oxidative stress; Ultrastructural

1. Introduction

A large controversy persists between blood and crystalloid cardioplegia about the benefits afforded to the heart by each of these procedures. Furthermore, differences between warm and cold blood cardioplegia add more confusion to determine which is the ideal method of myocardial protection. A randomized trial with 1000 patients, comparing warm blood vs. cold crystalloid cardioplegia failed to show any difference in the postoperative rates of myocardial infarction, death, or need for intraaortic balloon counterpulsation [1]. We believe that in order to compare two methods of myocardial protection, more sensitive determinations of myocardial damage are required. In this regard, we performed electron microscopy studies to assess ultrastructural changes. Considering that oxidative stress appears to play an

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important role in reperfusion injury, we also measured the antioxidant status and lipid peroxidation in blood samples obtained from the coronary sinus. Very few ultrastructural studies have been performed in humans submitted to open heart surgery [2–5], and there are several works showing the behavior of oxidative stress status in coronary sinus blood [6–11], or in myocardial tissue [12,13]. However, no works combining ultrastructural studies and myocardial oxidative stress in order to compare the degree of protection between warm blood and crystalloid cardioplegia have been reported.

Our study was designed to compare the degree of myocardial protection afforded by warm blood and cold crystalloid cardioplegia in a group of patients undergoing elective coronary artery bypass surgery. For this purpose, α-tocopherol, β-carotene, and ubiquinol, were assayed to measure changes in the antioxidant defensive system. To evaluate lipid peroxidation, thiobarbituric acid reactive substances (TBARS), were assayed. For all these chemical determinations, plasma samples were obtained from the coronary sinus. Ultrastructural damage was evaluated by electron microscopy studies of the left ventricular wall.

2. Material and methods

2.1. Patients

Seventeen patients submitted to coronary artery bypass surgery were randomized to group A ($n=9$), who received crystalloid cardioplegic solution, and group B ($n=8$) who received warm cardioplegic solution. Baseline characteristics of the two groups are depicted in Table 1. Written informed consent for participation in the study was obtained from each patient and the hospitals' ethical committee approved the protocol.

Table 1
Clinical data

	Group A ($n=9$)	Group B ($n=8$)
Age (years)	54±4	51±2
Ejection fraction (%)	53±3	53±2
Ischemic time (min)	53±5	49±5
Number of grafts	2.9±0.3	3.0±0.3

The presence of recent (less than 4 weeks) myocardial infarction; ejection fraction below 40%; associated valvular disease; history of antioxidants intake for the last 30 days before surgery, and type II diabetes, were considered as exclusion factors.

2.2. Operative techniques

Similar anesthetic agents were administered to all patients. Cardiopulmonary bypass was instituted with mild hypothermia between 32 and 33 °C. Before the aorta was clamped, and 10 min after reperfusion, biopsy and blood samples were taken (Fig. 1). Full-thickness biopsy samples were obtained from a normal area in the apex of the left ventricle or close to it in the anterior wall (Travenol Tru-Cut biopsy needle, Baxter Healthcare Corp., Valencia, CA, USA). Tissue specimens were immersed in cold 3% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4). Blood (10 ml) was obtained from a coronary sinus perfusion cannula previously introduced through a separate purse string suture in the right atrium and manually guided into the coronary sinus. The blood was immediately centrifuged and the plasma kept frozen and sent for chemical assays.

2.3. Administration of cardioplegia

Group A received immediately after clamping of the aorta, 500 ml of Saint Thomas cardioplegia at 4 °C. Approximately 300 ml of this infusion was administered into the aortic root at a pressure between 70 and 90 mmHg, the remaining 200 ml were administered into the coronary sinus at less than 40 mmHg of pressure. Every 20 min, an additional 300

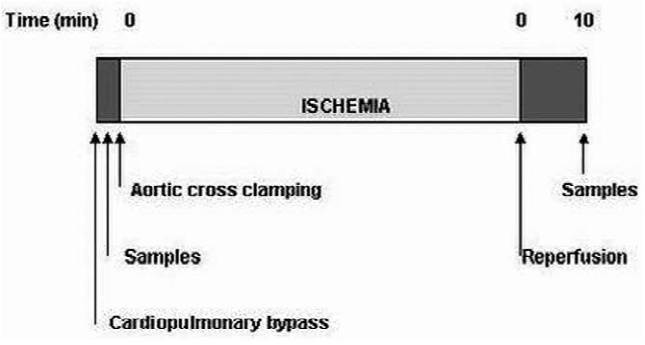

Fig. 1. Design followed in this trial.

ml of the same infusion was administered in the same fashion. Then, 5 min before the clamp was removed 300 ml of warm blood was administered. Each patient received an average of 1000 ml during the ischemic period.

Group B received warm (33–35 °C) blood containing 40 mEq/l potassium and hematocrit ranging between 20 and 25%, using the same method of delivery than for Group A.

2.4. Assays

2.4.1. Antioxidants

Standard solution of α-tocopherol was prepared by dissolving the pure compounds (Sigma, St. Louis, MO, USA) in methanol–ethanol, 50:50 (MeOH:EtOH) to yield final concentrations for approximately 10–50 μM. The accurate concentration for the standard solution was determined spectrophotometrically using the molar extinction coefficients for α-tocopherol, 292–294 nm=71–76. The standard was stable for several weeks when kept at −20 °C. A 200 μl-aliquot of plasma was mixed with 500 μl of methanol. The mixture was vortexed for 30 sec, and added with 4 ml of hexane, vigorously vortexed for 1 min and centrifuged for 5 min at 100×*g* to separate the phases. A 3 ml-aliquot of the hexane layer was transferred to another tube and dried under N_2. The residue was re-dissolved in 0.5 ml methanol–ethanol 1/1 (v/v) and filtered through a 0.22 μm-pore membrane. HPLC conditions: isocratic reversed phase HPLC; column:supelcosil LC-8, 3.3 cm×4.6 mm, 3 μm; precolumn:supelguard LC-8; mobile phase: 20 mM lithium perchlorate in methanol–water 99/1 (v/v); flow rate: 1 ml/min; retention time (min): α-tocopherol=0.8; electrochemical detection: oxidation potential +0.6 V; UV detection: 290 nm.

A similar procedure was carried on for β-carotene and ubiquinol determinations.

2.4.2. Lipid peroxidation products

TBARS are an evaluation of dialdehydes produced by free radical (FR) damage on cellular components, and malondialdehyde (MDA), a known product of the lipid oxidation, accounts for an important percentage of those aldehydes. Preparation of standard: Standard solution of MDA was prepared by acid hydrolysis of 1,1,3,3-tetrametoxypropane. The accurate concentration of the standard solution was determined spectrophotometrically using the molar extinction coefficients for MDA. The standard is stable for several weeks when kept at −20 °C. TBARS were determined using a fluorometric method to assay lipid peroxidation. TBARS were extracted into *n*-butanol and the fluorescence of the butanol layer was measured at 515 nm excitation and 555 nm emission. The values were expressed as micromoles of TBARS (MDA equivalents) per liter of human blood plasma.

2.5. Electron microscopy

Tissues to be used for transmission electron microscopy were fixed in cold 3% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated, end embedded in Epon. Three blocks were selected from different depths of each biopsy sample. From each block, a section 1 μm thick was cut, stained with 1% toluidine-borax, and examined by light microscopy to select appropriate areas for thin sectioning. Five ultrathin sections were obtained from each of the three blocks. Ultrathin sections were mounted in copper grids, stained with uranyl acetate and lead citrate, and examined with a Jeol Jem-100C, Japan electron microscope.

Electron micrographs were taken systematically at magnification of ×5000, so that a comparative evaluation of the preischemic and reperfusion biopsy samples from both groups could be done. These evaluations were also carried out in a blind manner by two different observers who assigned a single score to each of the areas based on the criteria of Kloner et al. [14]. A numerical value of 0 through 4 was assigned for each mitochondrion, depending on the degree of morphologic damage. The grading scale used to determine these values was as follows: 0, normal mitochondria; 1, early swelling as manifested by separation of cristae and clearing of matrix density; 2, more marked swelling than in grade 1; 3, massive swelling with architectural disruption; and 4, findings in grade 3 plus rupture of inner and outer mitochondrial membranes. The average obtained from the two observers was expressed for each grade as a percentage of the total number of mitochondria

counted per sample. Approximately 150 mitochondria per sample were graded in this manner.

Overall injury to myocardial cells was ranked as follows: normal, damaged (glycogen loss, nuclear chromatin clumping and margination, and I bands plus intermyofibrillar and sarcoplasmic reticular edema), and necrotic (subsarcolemmal blebs, sarcolemmal gaps, marked edema, loss of sarcomere structure, and disruption of mitochondria with osmophilic amorphous densities in their increased matrix space).

2.6. Statistical analysis

Results are presented as mean±S.E. unless otherwise indicated. Statistica for Windows, release 5.0 (StatSoft) was employed to performed the analysis ($P<0.05$ was considered significant). Comparisons of plasma determinations were made using the Tukey–Kramer Multiple Comparison test, and those assessing mitochondrial damage were made using Student's *t*-test for dependent samples.

3. Results

3.1. Clinical outcome

There were no major complications in both groups regarding myocardial infarction, mechanical support and death. Two patients in group A and one patient in group B required inotropic support for more than 2 h in the postoperative period.

3.2. Determinations of antioxidants

No significant changes was observed between preischemia and reperfusion samples in both groups concerning α-tocopherol, β-carotene and ubiquinol, and no differences between groups were detected. A slight rise in reperfusion values for the three antioxidants was observed without statistical significance (Table 2).

3.3. Determination of TBARS

A rise in TBARS was observed for both blood and crystalloid groups in reperfusion samples compared to the respective preischemia values. The difference was highly significant for group A samples (Table 2).

3.4. Electron microscopy studies

3.4.1. Qualitative analysis

The main morphologic findings in the presichemic samples consisted of sarcolemmal and intercalated disk preservation, mild mitochondrial edema, diffuse glycogen loss and sarcomere preservation and/or hyperconcentration and/or focal fragmentation. Also, mild cytosolic and intermyofibrillar edema, scarce t-tubules dilation, and nuclear chromatin margination were observed. Regarding postreperfusion samples, the same findings as in preischemic samples as well as moderate or severe mitochondrial edema, moderate or marked cytosolic and intermyofibrillar edema were observed, indicating higher myocardial damage (Figs. 2 and 3).

Table 2
Plasma determinations from coronary sinus for α-tocopherol, β-carotene, ubiquinol and TBARS

	α-Tocopherol	β-Carotene	Ubiquinol	TBARS
Preischemia GrA	27.84±4.18	0.31±0.09	0.79±0.1	3.49±0.3
	P=NS	P=NS	P=NS	$P<0.01$
Reperfusion GrA	26.37±2.7	0.42±0.14	0.99±0.14	5.2±0.45
Preischemia GrB	27.39±1.2	0.2±0.04	0.67±0.05	2.6±0.3
	P=NS	P=NS	P=NS	P=NS
Reperfusion GrB	27.48±2.2	0.21±0.04	0.62±0.04	3.54±0.3

Values are expressed as mean±standard error of the mean.
NS, not significant; GrA, Group A; GrB, Group B.
Significance determined by the Tukey–Kramer Multiple Comparison Test.

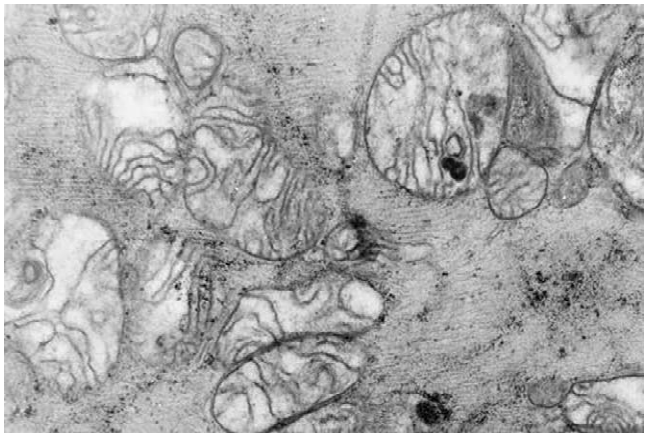

Fig. 2. Transmission electron micrograph of a reperfusion sample from the group A (crystalloid cardioplegia). Damage to mitochondria is severe, with massive swelling and disruption of cristae or marked swelling and rupture of membranes (original magnification, ×10 000).

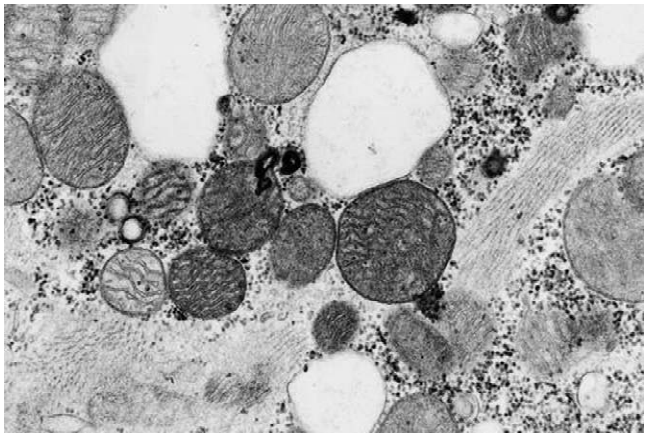

Fig. 3. Transmission electron micrograph of a reperfusion sample from the group B (warm blood cardioplegia). Mitochondria have intact membranes and tightly packed cristae (original magnification, ×10 000).

3.4.2. Quantitative analysis

Grading of mitochondrial damage: No significant difference was found between both groups in the grading of mitochondrial damage from preischemia biopsies. A significant decrease in grade 0 as well as a significant rise in grade 3 and 4 mitochondrial damage was observed in the reperfusion biopsy samples from group A compared to preischemia samples ($P=0.028$; $P=0.02$; $P<0.01$, respectively). The same trend of changes were present in group B but differences did not reach statistical significance except for grade 0 ($P=0.005$) (Table 3).

4. Discussion

In this paper, we found a significant rise in TBARS during reperfusion in plasma from coronary sinus in samples from group A as compared to preischemia samples. In group B TBARS were also increased during reperfusion but without reaching statistical significance. No differences in the sinus blood concentrations of antioxidants were observed in both groups between presichemia and reperfusion values.

Increased severely damaged mitochondria were present during reperfusion in both groups as compared to preischemia values, and this difference became significant in group A. A significant depletion of normal (grade 0) mitochondria was also observed in this group.

Myocardial preservation techniques have revolutionized cardiac surgery. Their main goal is to preserve the physical and chemical conditions of the tissue during the ischemic period in order to decrease

Table 3
Sermiquantitative determination of mitochondrial damage in both groups

		Grading of damage (%)					Number of checked mitochondria
		0	1	2	3	4	
Group A	P	65.1±7	18.6±3	9.8±3	4.1±1.3	2±0.78	1638
		$P=0.028$	NS	NS	$P=0.02$	$P<0.01$	
	R	36.9±10	13±2	19±4	21.2±6	9.4±3	1635
Group B	P	60±12	14.6±2	11±5.4	4.9±3.6	8±5.6	1325
		$P=0.005$	NS	NS	NS	NS	
	R	32.38±10	25.2±7.4	13.9±3	20±6	8±4	1419

P, preischemic samples; R, reperfusion samples.
Values are expressed as mean±standard error of the mean.
Significance determined by *t*-test for dependent samples.

reperfusion injury. This phenomenon, described by Hearse, determines that after a time of ischemia, ultrastructural damage to the heart increases during reperfusion [15]. This is associated with functional alterations of the myocardial contractility and is probably the main mechanism of myocardial stunning, which is observed in variable degree during the postoperative period.

Extensive research in the isolated heart and in the heart in situ in experimental models, and more recently, several studies in the human heart, support the free radical hypotheses as one of the mechanisms of reperfusion damage. Numerous studies have evidenced that during reperfusion, a burst of free radicals is generated in the previously ischemic tissue [16,17]. In the heart, this phenomena was extensively observed and the production of free radical peaks over 7-fold within the first minutes of reperfusion and continues at a lower rate for hours [18]. Oxygen radical generation as a consequence of postischemic reperfusion is undeniably linked to numerous papers by Zweier and Bolli. Zweier [19] showed that radical scavengers administered at time of reperfusion could both scavenge these radicals and improve functional recovery. This observation has led to many subsequent studies both from Zweier and others, including the classic experiments of Roberto Bolli showing the role of free radicals during the early seconds of reperfusion as mediators of myocardial stunning [20].

According to Ambrosio et al. [21,22], activated neutrophils constitute one of the main sources of oxidants in hearts after reperfusion. Inhibition of NADPH oxidase, an inducible enzyme largely responsible of this oxidative burst, produces a marked reduction of free radical activity.

Free radicals appear to affect calcium homeostasis, leading to a state of progressive calcium overload and its subsequent deleterious consequences over cell structures [17]. This should be the result of alterations produced by free radicals on the molecular structure of enzymatic channels related to calcium exchange at the level of the sarcoplasmic reticulum and at the sarcolemma [23]. The function of these enzymes is affected by oxidation of one or more thiol groups at the active center. The resulting disturbance upon the activity of these enzymatic channels is responsible for the early manifestations, such as arrhythmia, occurring during the first seconds after reperfusion. Immediately after, a second wave of damage is produced, when free radicals directly oxidize other biomolecules, particularly the fatty acids of cell membranes. The demonstration that lipid peroxidation is a hallmark of oxygen radical-mediated injury in reperfused hearts comes from the work of Ambrosio et al. [24].

Recently, overwhelming evidence indicates that nitric oxide plays a critical role in reperfusion and preconditioning. Specifically, enhanced biosynthesis of nitric oxide is essential to trigger the late phase of ischemia-induced preconditioning exerting a protective effect on the myocardium [25]. This may explain, at least in part, the beneficial effect of intermittent clamping technique performed by some cardiac surgeons.

Considering that free radicals appear to play an important role in reperfusion injury, it is reasonable to measure oxidative stress as a marker of myocardial damage and hence as a parameter to evaluate the protective action of a determined cardioplegic solution. In previous works, we used chemiluminescence from tissue biopsies, and it was demonstrated for the first time, the production of an oxidative burst during reperfusion in humans [2]. An alternative to study oxidative stress is to measure TBARS in plasma obtained from the coronary sinus. Through the same source, we also evaluated the behavior of the antioxidant defense system of the heart submitted to the ischemia–reperfusion mechanism. For this purpose α-tocopherol, β-carotene, and ubiquinol were assayed.

We did not observe a significant depletion of antioxidants in either group during reperfusion. These results are in disagreement with some authors who observed decreased levels of glutathion [26] and vitamin E [27] from coronary sinus during reperfusion after angioplastic procedures. On the other hand, Kim et al. [13] did not detect significant changes in glutathion levels from biopsy samples before ischemia and during reperfusion. In this experience TBARS appeared to be a more reliable method than antioxidants to measure oxidative stress after ischemia.

In spite of an absence of antioxidants depletion in reperfusion samples, the presence of increased lipid peroxidation strongly suggest that a free radical burst is at least partially responsible for the ultrastructural

changes in myocardial cells [28,29]. In this regard, previous works have shown reduced damage and improved outcome after surgery with the preoperative administration of antioxidants [30–33], or supplementing the cardioplegic solution with these substances [3], or with iron chelating agents [4,34].

Semiquantitative determinations of mitochondrial damage clearly showed that blood cardioplegia offers better protection to the myocyte than cold crystalloid cardioplegia. This is of particular value because ultrastructural studies constitute a direct and strong way to evaluate the status of the myocardium.

According with this study, blood cardioplegia appears to offer better protection to the myocyte than crystalloid cardioplegia.

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