Relationship between oxidative stress, lipid peroxidation, and ultrastructural damage in patients with coronary artery disease undergoing cardioplegic arrest/reperfusion

José Milei, Pedro Forcada, César G. Fraga, Daniel R. Grana, Gabriele Iannelli, Massimo Chiariello, Isabella Tritto, Giuseppe Ambrosio

Abstract

Objective: In animal models, formation of oxidants during postischemic reperfusion may exert deleterious effects (“oxidative stress”). Cardioplegic arrest/reperfusion during cardiac surgery might similarly induce oxidative stress. However, the phenomenon has not been precisely characterized in patients, and therefore the role of antioxidant therapy at cardiac surgery is a matter of debate. Thus, we wanted to ascertain whether the relationship between oxidant formation and development of myocardial injury also translates to the situation of patients subjected to cardioplegic arrest.

Methods: In 24 patients undergoing coronary artery bypass, trans-cardiac blood samples and myocardial biopsies were taken before cardioplegic arrest and again following reperfusion.

Results: Cardiac glutathione release (marker of oxidant production) was negligible at baseline (0.02±0.04 μmol/L), but it increased 15 min into reperfusion (1.10±0.40 μmol/L; p<0.05); concomitantly, myocardial concentration of the antioxidant ubiquinol decreased from 144.5±52.0 to 97.6±82.0 nmol/g (p<0.05). Although these changes document cardiac exposure to oxidants, they were not accompanied by evidence of injury. Neither coronary sinus blood nor cardiac biopsies showed increased lipid peroxide concentrations. Furthermore, electron microscopy showed no major ultrastructural alterations. Finally, full recovery of left ventricular systolic and diastolic function was observed.

Conclusions: Careful investigation reveals that while oxidant production does occur during cardiac surgery in patients with chronic ischemic heart disease, cardiac oxidative stress may not progress through membrane damage and irreversible injury.

Keywords: Oxidative stress; Ischemia; Reperfusion; Coronary artery by-pass graft

This article has been referred to in the Editorial by D.J. Chambers (pages 626–628) in this issue.

Reintroduction of blood to hearts subjected to ischemia may induce a condition of “oxidative stress” due to formation of oxygen radicals and other “reactive oxygen species” (ROS), which might result in cell injury. During cardiac surgery, the heart is kept arrested for a substantial amount of time and then subjected to...
reperfusion. Because this condition has evident analogies with posts ischemic reperfusion, the possibility that ROS-mediated injury may develop during cardiac surgery has attracted obvious interest. Preventing this phenomenon would improve myocardial protection during surgery and postoperative outcome. However, despite encouraging results initially obtained in animal models [3–5], clinical trials of antioxidants in cardiac surgery have yielded conflicting results [6–15]. This might stem from incomplete characterization of the phenomenon in patients, which might lead to non-optimal design of antioxidant strategies.

Formation of ROS during posts ischemic reperfusion triggers a complex chain of events [1,2]. During cardiac surgery, ROS formation does occur during reperfusion after cardioplegia, as shown by direct measurement of radicals [16–18] and of cardiac release of glutathione (a marker of oxidant formation) [19]. However, while these findings clearly demonstrate exposure to oxidants, they do not necessarily equate injury. Glutathione is an endogenous mechanism of oxidant inactivation [20,21]; accordingly, once formed ROS oxidize glutathione (which is then released outside the cells [22]) and are inactivated in the process. Up to a substantial amount, this effectively prevents further spreading of oxidative damage. If oxidant attack continues and/or intensifies, oxidation of lipid constituents of membranes ensues, which impairs the function of cell organelles and eventually culminates in ultrastructural injury [23]. While this sequence of events has been clearly documented in experimental models [24–27], evidence for the occurrence of this phenomenon in patients subjected to cardiac surgery is scanty. Furthermore, it has not been gathered in a comprehensive fashion so as to link the various alterations together and to provide an encompassing picture of the phenomenon.

In the present study, we investigated whether a precise relationship exists between the occurrence of oxidative stress, peroxidation of membrane lipids, and ultrastructural evidence of irreversible tissue injury in the heart of patients with chronic ischemic heart disease undergoing ischemia/reperfusion at cardiac surgery.

### 1. Methods

#### 1.1. Patient selection

Twenty-four consecutive patients scheduled for elective coronary artery bypass surgery at Hospital Francés, Buenos Aires, were enrolled. Inclusion criteria were: a) stable (>3 months) exercise-induced angina; b) ≥70% stenosis of the left anterior descending coronary artery and at least one other major vessel, suitable for bypass surgery; c) ejection fraction >40%; d) no recent (>4 weeks) acute coronary syndrome; e) written informed consent. Patients with valvular disease or treated with allopurinol, ACE-inhibitors, or antioxidant drugs were excluded. The study protocol was approved and controlled by the Ethics Committee of the Argentine Society of Cardiology. The investigation conforms with the principles outlined in the Declaration of Helsinki.

#### 1.2. Study protocol

##### 1.2.1. Inclusion visit

At day 0, eligible patients underwent clinical examination, ECG, and evaluation of LV systolic and diastolic function by radionuclide angiography. Therapy was adjusted, if needed (Fig. 1).

##### 1.2.2. Presurgery visit

20 days after inclusion visit, patients were admitted. Clinical examination, ECG, and radionuclide angiography were repeated. Beta-blockers were discontinued.

##### 1.2.3. Discharge visit

Ten days after surgery, i.e. at the time of, or immediately after discharge, clinical examination and radionuclide angiography were repeated.

#### 1.3. Radionuclide angiography

Autologous Tc99-labeled erythrocytes were administered. Acquisitions were performed with a gamma-camera

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### STUDY PROTOCOL

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Fig. 1. Study protocol. Blood sampling: venous and coronary blood sampling. Biopsies: TBARS, alpha-tocopherol, beta-carotene, ubiquinone, pathology.
interfaced to a computer using ECG gating. Sixteen frames/\(R\)–\(R\) interval were taken to assess LV wall motion and ejection fraction. Acquisitions were analyzed for peak filling rate, time-to-peak filling, ejection fraction, and regional function, by an investigator unaware of clinical conditions of patients.

1.4. Surgical protocol

Patients were sedated, a radial artery was cannulated, and a Swan-Ganz catheter advanced into the pulmonary artery. Arterial blood pressure, heart rate, right atrial pressure, pulmonary artery pressure, cardiac output, stroke work index, and ECG, were measured. Anesthesia was achieved with administration of fentanyl, benzodiazepine, panceuronium bromide. After sternotomy, cardiopulmonary bypass was instituted under mild hypothermia (32–33 °C). Following aortic clamping, the heart was arrested by intraaortic infusion of 500 mL of cold (4 °C) St. Thomas cardioplegic solution, delivered at a pressure of 80 mm Hg. A perfusion cannula was introduced through a purse-string suture in the right atrium and advanced into the coronary sinus, for retrograde delivery of 100 mL cardioplegic solution (at 4 °C) every 30 min, and for sampling of cardiac venous effluent. The left internal mammary artery was used to revascularize the left anterior descending coronary artery; saphenous vein grafts were used for lesions of the circumflex and/or the right coronary artery. After completion of the grafts, the aortic clamp was removed and the heart reperfused.

1.5. Blood and tissue sampling

Blood samples were taken simultaneously from coronary sinus and from the arterial line before clamping, and 5 and 20 min after reperfusion. After centrifugation plasma was frozen and stored for biochemical assays. Full-thickness biopsies (\(\approx 20 \text{ mg}\); Travenol Tru-Cut needle, Baxter Corp., Valencia, CA) were taken from the anterior wall of the LV near the apex, immediately before inducing cardiac arrest, and 10 min after reperfusion [28]. Direct inspection confirmed that wall motion was normal, and no scar tissue was present at biopsy site. Biopsies were fixed for electron microscopy (see below), or homogenized for biochemical assay (see below). CK-MB concentration was measured (Wiener, Rosario, Argentina) on peripheral blood collected before surgery, 4 h after reperfusion, and 4 days postoperatively.

1.6. Biochemical determinations

1.6.1. Tissue homogenization

Tissue biopsies were homogenized in 10 vol of 120 mM KCl, 30 mM K3PO4, pH 7.4, in the presence of 1 vol of butylated hydroxytoluene (BHT, 4% w/v in ethanol). The suspension was centrifuged at 600 \( \times g \) for 10 min to discard nuclei and cell debris, and the supernatant used as "tissue homogenate".

1.6.2. Antioxidants

Vitamin E (as \( \alpha \)-tocopherol) and ubiquinone (as ubiquinol-10) were determined by HPLC with electrochemical detection [29,30]. Briefly, 100 \( \mu \)L-aliquots of tissue homogenate were mixed with 500 \( \mu \)L of methanol, vortexed for 30 s, added with 4 mL of hexane, vortexed for 1 min, and centrifuged for 5 min at 1000 \( \times g \) to separate the organic phase. A 3 \( \mu \)L-aliquot of the hexane phase was transferred to another tube and dried under \( N_2 \). The residue was redissolved in 0.5 mL methanol/ethanol 1/1 (v/v) and filtered through a 0.22 \( \mu \)m-pore nylon membrane. The filtrate was separated by isocratic HPLC using a Supelcosil LC-8, 3.3 cm \( \times \) 4.6 mm column, with a 3 \( \mu \)m Supelguard LC-8 precolumn. The mobile phase, 20 mM LiClO4 in methanol/water 99/1 (v/v), was pumped isocratically at 1 mL/min. \( \alpha \)-Tocopherol was determined amperometrically at +0.6 V and spectrophotometrically at 290 nm [29]. The same HPLC and detection conditions were used for determining ubiquinol-10: in this case, sample preparation required prior chemical reduction of oxidized ubiquinol (ubiquinone) to ubiquinol. The filtrate was added with catalytic amounts of sodium borohydride, reextracted with hexane and processed as for \( \alpha \)-tocopherol [30]. Standard solutions of \( \alpha \)-tocopherol and ubiquinol-10 (10–50 \( \mu \)mol/L) were prepared by dissolving pure compounds (Sigma Chem Co, St. Louis, MO) in methanol/ethanol 50/50. Concentrations were determined spectrophotometrically using the molar extinction coefficients 292–294 nm = 71–76.

1.6.3. Glutathione

For determination of glutathione, 500 \( \mu \)L of plasma were deproteinized with 10 volumes of 0.33 mol/L of HClO4. After neutralization of the supernatant with 1.75 mol/L of K3PO4, an aliquot was taken and glutathione concentration measured using the glutathione reductase-DTNB ‘5,5’-dithiobis(2-nitrobenzoic acid) spectrophotometric assay [22].

1.6.4. Lipid peroxidation

Di-keto compounds produced by oxidation of lipids were determined fluorometrically as thiobarbituric acid-reactive substances (TBARS) in tissue homogenates (adjusted to 50 \( \mu \)g of protein) or plasma samples (200 \( \mu \)L) [31]. Samples were added with 100 \( \mu \)L of BHT (4% w/v in ethanol) and then assayed. Results for tissues were expressed as nmol of TBARS (malondialdehyde equivalents)/mg protein, and for blood as \( \mu \)mol/L.

1.7. Ultrastructure analysis

Biopsies were fixed in cold 3% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4), postfixed in 1% OsO4, dehydrated, and embedded in Epon [28]. One \( \mu \)m-thick sections were cut, stained with 1% toluidine-borax, and examined by light microscopy. Ultrathin sections (15 for each biopsy) were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined under a Jem-100C (JEOL, Japan) electron microscope.
Electron micrographs were taken systematically at ×5000 magnification. Images were analyzed by two investigators unaware of sequence of sampling (i.e., preischemic or postreperfusion) and of clinical data of patients. Overall myocardial cell injury was ranked on a 0–4 scale as [32]: 0=normal; 1=minimal ischemic (glycogen loss, nuclear chromatin clumping/margination); 2=moderate changes (as grade 1, plus intermyofibrillar and sarcoplasmic reticulum edema); 3=severe changes (as grade 2, plus subsarcolemmal blebs, sarcolemmal gaps, marked edema; and, 4= complete architectural disruption and nuclear lysis. Cells scoring 3 and 4 were considered necrotic. Damage of each mitochondrion was scored on a 0–4 scale, according to the extent of morphological alterations, as [32]: 0=normal; 1=initial swelling (separation of cristae, decreased matrix density); 2=more marked swelling than grade 1; 3=massive swelling with architectural disruption; and, 4= findings as grade 3 plus rupture of mitochondrial membranes. Average value from two observers was expressed as percentage of total number of mitochondria counted per sample. Approximately 150 mitochondria/sample were graded.

1.8. Additional investigations

Based on the results of lipid peroxidation (see Results), we expanded our analysis to biopsies deriving from a concomitant study being conducted in Naples, Italy, in which we enrolled 8 consecutive patients with: a) mitral or aortic valve disease requiring elective replacement; b) no angiographic evidence of coronary stenosis; c) normal LV function. Data from this second arm were kept separate, and employed to specifically address the issue of preferential peroxidation of cellular organelles. After sternotomy, cardiopulmonary bypass was instituted under mild hypothermia (32–33 °C). The aorta was clamped and the heart arrested by intraaortic infusion of 500 mL of cold (4 °C) St. Thomas cardioplegic solution. After valve replacement, the aortic cross-clamp was removed and the heart reperfused. Full-thickness myocardial biopsies (20–25 mg; Travenol Tru-Cut) were taken immediately before cardioplegic arrest and 10 min after reperfusion, from the anterior LV wall near the apex. Biopsies were homogenized and subjected to sequential centrifugations to separate different organelles, as identified by presence of specific marker enzymes (for details [26,33]). The whole homogenate and the various subcellular fractions were assayed for peroxidation products content by a modified thiobarbituric acid (TBA) method [26,33,34].

1.9. Statistical analysis

Statistical analysis was performed using Statistica-for-Windows software. Continuous variables were compared by 2-tailed paired t-test. One-way ANOVA was employed for multiple determinations, followed by Tukey–Kramer test. In case of categorical variables, chi-square with Yates correction was applied; with gradual variables or scales, Wilcoxon rank-test was used. In case of interrelated variables, Spearman rank-order correlation test was employed. Differences in mitochondrial scores were compared by two-way ANOVA.

2. Results

2.1. Patient characteristics

Table 1 shows patients clinical and demographic characteristics. All patients underwent successful revascularization. Aortic cross-clamp averaged 40.9±11.9 min. All patients recovered satisfactorily from surgery, without inotropic support. Plasma CK-MB concentration increased from 2.4±1.7 IU/L to 11.5±6.8 IU/L (p<0.01) 4 h after surgery, and declined to 6.5±2.9 IU/L at day 4. Two patients died, one 24 h postoperatively (acute myocardial infarction), and one 28 days after surgery (mediastinitis), and were excluded from the analysis.

2.2. Cardiac function

The surgical procedure had only modest and transient effects on cardiac function. Hemodynamic parameters returned to normal within a few hours postoperatively (not shown). Ejection fraction and peak filling rate remained unchanged (Table 2); time-to-peak filling rate at discharge

<table>
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<th>Enrollment</th>
<th>Preoperative</th>
<th>Postoperative (10 days)</th>
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<tr>
<td>LVEF (%)</td>
<td>53.1±8.5</td>
<td>51.9±9.0</td>
<td>52.5±10.7</td>
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<td>Peak filling rate (EDV/s)</td>
<td>1.7±0.7</td>
<td>1.7±0.5</td>
<td>1.6±0.7</td>
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<tr>
<td>Time-to-peak filling (ms)</td>
<td>195.3±123.5</td>
<td>193.1±85.7</td>
<td>166.4±100.9</td>
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Data are mean±SD. EDV=end-diastolic volume. LVEF=left ventricular ejection fraction.
was shorter than baseline, but the difference was not significant (Table 2).

2.3. Markers of oxidative stress in cardiac effluent

2.3.1. Glutathione

Before ischemia, concentrations of glutathione were similar in systemic arterial blood and in blood from the coronary sinus (0.7 ± 0.2 and 0.8 ± 0.7 μmol/L, respectively). During reperfusion after cardioplegic arrest, glutathione concentration in arterial blood entering the heart remained stable (peak 0.8 ± 0.7 μmol/L); in contrast, glutathione concentration in blood drawn from the coronary sinus significantly increased (1.8 ± 0.2 μmol/L; \( p < 0.01 \)); thus, a net trans-cardiac release of glutathione (i.e., positive difference between venous minus arterial concentrations) occurred at 5 min reperfusion, which persisted after 20 min (Fig. 2).

2.3.2. Lipid peroxides

Prior to cardiac arrest, thiobarbituric acid-reactive substances (TBARS) in systemic arterial blood averaged 3.7 ± 0.6 μmol/L; concentration in venous blood from the coronary sinus was slightly lower than in arterial blood (3.3 ± 0.9 μmol/L), indicating a tendency to cardiac extraction of lipid peroxides (Fig. 3) consistent with metabolism by mitochondria aldehyde-dehydrogenase and aldose-reductase [35,36]. Five min after reperfusion, TBARS concentrations were similar in arterial blood entering the heart (3.1 ± 0.6 μmol/L), and in coronary sinus blood (3.1 ± 0.7 μmol/L); therefore, no net cardiac release was observed (Fig. 3); this behavior persisted at 20 min of reperfusion (Fig. 3).

2.4. Markers of oxidative stress in cardiac tissue

2.4.1. Antioxidants

Cardiac concentration of ubiquinol decreased >30% as an effect of ischemia/reperfusion, from 144.5 ± 52.0 nmol/g in biopsies taken before cardioplegic arrest, to 97.6 ± 82.0 nmol/g 10 min after reperfusion (\( p = 0.05 \)). Concentration of \( \alpha \)-tocopherol did not change, averaging 42.6 ± 28 nmol/g in preischemic biopsies, and 46.7 ± 25 nmol/g in biopsies taken after postischemic reperfusion.

2.4.2. Lipid peroxides

TBARS averaged 0.99 ± 0.42 nmol/mg protein in biopsies taken before cardioplegic arrest, and 0.92 ± 0.37 nmol/mg protein in biopsies taken 10 min after reperfusion (\( p = \text{NS} \)). Thus, no increase in membrane lipid peroxidation was documented in the whole cardiac extract.

Previous animal studies of oxygen radical-mediated reperfusion injury had shown that peroxidation of membrane lipids can be prominent in specific subcellular organelles [25,26,33]. To investigate this specific point, we analyzed cardiac biopsies that were processed to isolate various subcellular fractions in a concomitant study. Also in this case, there was no increase in TBARS after postischemic reperfusion, neither in the whole homogenate, nor in any subcellular fraction (Table 3).

2.5. Electron microscopy

2.5.1. Qualitative analysis

Intracellular myocyte architecture was preserved in preischemic biopsies (Fig. 4). Sarcomere alignment was preserved; sarcocellular membrane, T-tubules, intercalated disks, and basement membranes were intact; nuclei and cytoplasmic glycogen were generally well preserved (Fig. 4, left panel); mild cytosolic and mild intermyofibrillar edema

![Fig. 2. Transcardiac glutathione release before cardioplegic arrest, and at 5 and 20 min reperfusion. Data are coronary sinus minus aorta concentrations. \(*=p<0.05\) vs pre-ischemia.](image)

![Fig. 3. Transcardiac release of lipid peroxides before cardioplegic arrest, and at 5 and 20 min reperfusion. Data are coronary sinus minus aorta concentrations. \(p=\text{NS}\).](image)

<table>
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<th>Subcellular distribution of lipid peroxidation products</th>
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<tr>
<td>Specimen</td>
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</tr>
<tr>
<td>Whole homogenate</td>
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<tr>
<td>Mitochondria</td>
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<tr>
<td>Cytosol</td>
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<td>Reperfusion</td>
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Data are mean ± SD of nmol/mg prot. \( p = \text{NS} \).
was occasionally seen; mitochondria showed tightly packed cristae, with no or minimal signs of edema (Fig. 4, right panel).

Cardioplegic arrest/reperfusion did not result in major morphological alterations. Overall myocardial and vascular structure was generally preserved (Fig. 5-A). Reperfusion biopsies mostly showed areas with intermyofibrillar edema and decreased or absent glycogen stores (Fig. 5-B); mitochondria mostly showed mild-to-moderate damage (Fig. 5-B). In some areas, moderate mitochondrial swelling, with decreased matrix density and separation of cristae, mild sarcomere hypercontraction, and dilatation of T-tubules, were observed (Fig. 5-C). Massive mitochondrial swelling and myofibrillar alterations were seen occasionally (Fig. 5-D). Severe changes (e.g., subsarcolemmal blebs, sarcolemmal gaps) were rare, and complete architectural disruption and nuclear lysis extremely scarce.

2.5.2. Quantitative analysis

Overall injury score showed no differences between preischemic and reperfusion biopsies (1.7 ± 0.5 vs 1.8 ± 0.4). In addition, since mitochondria can be a target of reperfusion injury, systematic evaluation was performed scoring 150 mitochondria/section (i.e., >2000 mitochondria/biopsy).

Fig. 4. Typical electron microscopy appearance of myocardial biopsies before cardioplegic arrest. Left panel: normal mitochondria, mild cytosolic and mild intermyofibrillar edema, and modest T-tubules dilation can be observed. ×8000. Right panel: cross-section at higher magnification, showing mitochondria with intact membranes and tightly packed cristae. ×10,000.

Fig. 5. Typical appearance of biopsies taken after 10 min reperfusion following cardioplegic arrest. Panel A. Large-field view by light microscopy of 1 μm-thick section. Myocardial architecture and vessel structure are well preserved. Mild intermyofibrillar edema is observed. 1% Toluidine-borax staining; ×400. Panel B. Transmission electron micrograph of reperfusion biopsy. Intermyofibrillar edema with mild sarcomere hypercontraction, glycogen decrease, and mild cytosolic edema. Mild mitochondrial damage (degree 0–2); ×8000. Panel C. Sarcomere hypercontraction, mitochondrial swelling, damage degree 1–2, and intermyofibrillar edema; ×10,000. Panel D. Focal sarcomere fragmentation is shown. Mitochondrial damage degree 3, lipid vacuoles, T-tubules dilation and myofibrillar disorganization, with glycogen loss; ×8000.
Before cardiac arrest, the great majority of mitochondria showed no or minimal alterations (Fig. 6). After reperfusion, there was a decrease in the number of normal mitochondria with concomitant increase in the proportion of mitochondria showing ultrastructural alterations (Fig. 6). This tendency, however, did not reach statistical significance ($p=0.08$).

### 3. Discussion

Experimental studies have documented ROS formation upon reperfusion of ischemic myocardium, with consequent oxidative injury to cell function and structure [1,2]. In the present study, in patients with coronary artery disease undergoing cardiac surgery we did find evidence of cardiac oxidant formation during reperfusion after cardioplegic arrest. However, this phenomenon did not progress into attack to cardiac membranes, nor did it translate into full-fledged ultrastructural injury. Finally, no impairment of cardiac function was observed. Together, these data demonstrate that no major oxidative injury occurs at reperfusion in the heart of patients with coronary artery disease subjected to cardiac surgery under the conditions of our study.

In most cardiac surgery procedures, the heart is kept arrested for a substantial amount of time and subsequently undergoes reperfusion: this condition is expected to favor oxidant formation. Indeed, ROS production occurs in patients during reperfusion after cardioplegic arrest [16–18], and is reduced by antioxidants/scavengers [12,15,37]. Yet, antioxidant strategies did not consistently translate into clinically relevant improvement [9–15]. An explanation for these controversial findings might be that the magnitude of oxidant-mediated injury may actually be modest under the conditions encountered in cardiac surgery. The findings of the present study lend credence to this hypothesis.

Consistent with previous reports [19], we observed transcardiac release of glutathione during reperfusion. However, although cardiac formation and release of glutathione is induced by oxidants [22,38,39], and consistently found during postischemic reperfusion [40,41], this may be simply an indication of exposure to ROS. It is only when defense mechanisms are overwhelmed that oxidant attack progresses to injuring cells. In fact, oxidant concentrations capable to elicit cardiac glutathione release larger than what found in this and previous clinical studies, have no detrimental effects, which nonetheless can be induced by up-titration of oxidants [38,39,42].

Endogenous antioxidants are another defense mechanism. In our patients, cardiac concentrations of ubiquinol decreased upon reperfusion, consistent with the notion that endogenous antioxidants are “consumed” in the process of oxidant detoxification [11,12,43]. This finding confirms that the hearts were exposed to ROS. However, since oxidation of glutathione and antioxidant vitamins occurs upstream in the chain of events following ROS formation, in order to establish whether injury has occurred these measurements must be complemented by other indices of oxidant attack.

Lipid oxidation is a major harmful consequence of ROS formation [23–26], as it reflects irreversible oxidative changes of membranes. Increased venous concentration of malondialdehyde has been found in patients subjected to cardiac surgery [10,11,44]. However, malondialdehyde concentrations in systemic blood may reflect changes unrelated to cardiac oxidative stress (prostanoid synthesis [45], activity of aldehyde-dehydrogenase [35] and aldose-reductase [36]). Indeed, surgery itself (independent of cardioplegic arrest/reperfusion) may induce systemic lipid peroxidation [18,44,46]. To specifically investigate lipid peroxidation at cardiac level, we simultaneously measured lipid peroxides in the blood entering the heart and in the coronary sinus effluent, and therefore could establish that no cardiac release of lipid peroxides occurred.

In experimental studies, lipid peroxidation of cardiac membranes was found at postischemic reperfusion [24–26], which was preventable by scavenging of ROS [26]. It can be taken as a reliable “chemical signature” of oxidative injury [24]. However, we did not detect any appreciable increase in lipid peroxide content of cardiac tissue, in spite of biopsies being taken 10 min after reinstitution of perfusion, when lipid peroxidation peaks [24]. To ascertain whether peroxidation may had occurred only in specific organelles, which might have gone undetected because of possible “dilution” within the whole homogenates, we also analyzed cardiac membranes isolated by subcellular fractionation. While this procedure does detect oxygen radical-mediated lipid peroxidation of specific organelles in ischemia/reperfusion models [25,26,37], it failed to document increased lipid peroxidation in our patients. In this study, lipid peroxidation was evaluated by measuring thiobarbiturate-reactive aldehydes. This approach has potential limitations in terms of specificity and possible artifactual formation. However, this is unlikely to have influenced our results since, if anything, it would have resulted in lipoperoxide concentrations being spuriously higher, whereas we found no increase at all. Furthermore, we had previously shown that under our conditions of...
homogenization and assay, malonyldialdehyde measurements compare favorably with other markers of cardiac membrane peroxidation [26,33].

Collectively, lack of lipid peroxide release, absence of increased membrane peroxidation, and preserved cardiac concentration of membrane-bound antioxidant α-tocopherol, all coincide to indicate that oxidant formation in our patients did not translate into appreciable oxidative modification of cell structures. To our knowledge, only one study has previously addressed this issue in a rigorous fashion: similar to what we have found, Janssen et al. [42] clearly documented that human hearts subjected to cardioplegic arrest/reperfusion release glutathione, but lipid peroxidation did not increase. However, that study was performed on explanted human hearts perfused ex vivo. Thus, our present study for the first time provide a thorough assessment of this issue in patients.

Ultrastructural alterations that characterize reperfusion injury (contraction-band necrosis, massive mitochondrial swelling, sarcolemmal disruption) were mostly absent. The finding that myocyte ultrastructure was largely preserved helps explaining why recovery of systolic and diastolic function was unimpeded, and it is internally consistent with absence of biochemical “signature” of irreversible oxidative injury in these patients. This lack of major alterations needs to be confronted with the results of earlier observations [47,48]. In one study [47], reperfusion biopsies showed decreased succinate-dehydrogenase activity (indicating loss of mitochondrial integrity), increased hydroperoxide-initiated chemiluminescence (marker of enhanced susceptibility to lipoperoxidation), and ultrastructural alterations; similarly, Weisel et al. [48], reported transcardiac release of conjugated dienes (a specific marker of lipid peroxidation), and decreased myocardial concentrations of membrane-bound α-tocopherol. However, several features of the present study may have contributed to the difference observed. First, since the ‘80s major advances have been made with respect to cardiopulmonary bypass technique and cardioplegia, all resulting in improved myocardial protection. For example, retrograde delivery of cardioplegic solution was not used then [47,48]. Furthermore, aortic cross clamp time averaged 40.9±11.9 min in this study, whereas it ranged between 55±5 min and 75±6 min then [47,48]; this may be important, since oxidative stress increases with length of arrest [19]. Finally, in the present study we investigated only patients scheduled for elective surgery, in stable clinical conditions, with good LV function; care was also exercised to optimize preoperative conditions, by adjusting patients’ medications three weeks before surgery.

In addition, it should be noted that in experimental studies ischemia/reperfusion is induced in healthy animals, while we studied patients with multivessel chronic ischemic heart disease. It is expected that most such patients had gone through brief ischemia/reperfusion episode(s) before cardiac surgery, and it is now established that sublethal ischemia may paradoxically “precondition” the heart, i.e., render it more tolerant toward a subsequent major insult of ischemia/reperfusion [49–52]. With preconditioning there may be upregulation of antiapoptotic proteins, activation of prosurvival kinases, and improved coronary perfusion with bypass grafting [49–52]. Thus, the possibility exists that hearts from patients with chronic ischemic heart disease may be “preconditioned”, and hence more resistant to subsequent injury by cardioplegic arrest/reperfusion than healthy, ischemia naive animals.

3.1. Implications

It is tempting to speculate that if the extent of oxidant injury is modest, there might be more harm than good in trying to eradicate oxidant production. Small amounts of oxidants may in fact precondition the heart, i.e., render it more tolerant toward a subsequent ischemia/reperfusion insult [50,53]. This phenomenon may occur during cardiac surgery [17,49,51,52], and the majority of studies of preconditioning in cardiac surgery have shown that ischemic preconditioning is an effective adjunct to myocardial protection in cardiac surgery [49]. Patients receiving antioxidants prior to and/or during cardiac surgery might thus be deprived of this potential benefit, while not gaining much from reduction of an otherwise small oxidant load at reperfusion.

3.2. Limitations

It is now appreciated that many pathological conditions, which are frequently present in patients with coronary artery disease (e.g., obesity, hypertension, diabetes, and hyperlipidemia) may induce oxidant stress and affect antioxidant defense systems, thereby modulating resistance to ischemia/reperfusion injury via a preconditioning effect [48–54]. On the other side, some drugs, such as ACE-inhibitors and angiotensin II type-1 receptor blockers, may inhibit oxidant stress and exert cardioprotective effects through the activation of eNOS [54]. Although we excluded patients taking these drugs from the study, our data do not allow to evaluate whether and how the complex pathophysiological relationship between oxidative stress and preconditioning was altered by the pathological conditions and events occurring in patients.

Because of protocol design centered around biochemical and ultrastructural parameters, and not based on clinical end-points, and because of the limited sample size, our findings cannot be taken as immediate evidence against administration of antioxidants in cardiac surgery. Furthermore, because of the strict inclusion criteria, our observations may not be applicable to all patients. Excluded are patients hemodynamically or clinically unstable; similarly, severely impaired ventricles might be more vulnerable to cardioplegic arrest/reperfusion; finally, procedures requiring longer cardioplegic arrest might be associated with greater extent of oxidative stress.
In conclusion, patients subjected to elective bypass surgery undergo oxidative stress upon reperfusion after cardiopulmonary arrest; the magnitude of the phenomenon, however, may be small and without major consequences. Our data indicate that devising optimal antioxidant therapy in the setting of cardiac surgery may require a more detailed understanding of the complex mechanisms of redox regulation in patients.

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References


