

Mitochondrial oxidative and structural damage in ischemia-reperfusion in human myocardium. Current knowledge and future directions

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1. ABSTRACT

The sequence of events in heart ischemia-reperfusion has been clearly documented in experimental animal models but not in cardiac surgery patients. The evidence in human studies had not been gathered in a systematic and comprehensive fashion, so as to provide an encompassing picture of the phenomenon. This limits our ability to devise appropriate strategies for optimal perioperative myocardial protection. We present here a review of our experience in myocardial ischemia-reperfusion in a historical perspective. From our previous studies we conclude that, although several issues still remain unsolved, there is no doubt that oxygen-free radicals are important contributors to myocardial injury during the reperfusion period of coronary artery bypass surgery. Yet, in spite of this wealth of information, both clinical and experimental, subsequent clinical trials conducted over the last several years with a variety of antioxidant strategies have been largely disappointing. Therefore, the whole paradigm of oxidative stress in cardiac injury needs to be re-evaluated. In this regard, differences between past and current knowledge are discussed, and future directions are traced. We concluded that patients subjected to elective bypass surgery undergo oxidative stress upon reperfusion after cardioplegic arrest; the magnitude of the phenomenon, however, is at present small and may not justify widespread antioxidant therapy.

2. INTRODUCTION

Heart ischemia-reperfusion injury is a complex phenomenon consisting of biochemical and ultrastructural alterations in myocardial cells which in turn may lead to electrical and contractile heart dysfunctions that are mainly expressed as myocardial stunning and reperfusion arrhythmias (1,2). Almost twenty years ago, a study from our group provided the first evidence of oxidative stress in the human heart during reperfusion associated with ultrastructural damage and suggested that oxygen free radicals could be involved (2). In that trial, oxidative stress was assayed as hydroperoxide-initiated chemiluminescence and mitochondrial damage was determined by electron microscopy, both in pairs of biopsies obtained before ischemia and after reperfusion. The study concluded that “these observations seem to indicate the presence of oxidative stress during reoxygenation, a situation that may play a major role in the genesis of reperfusion injury” (2).

It is now established that re-introduction of blood to hearts subjected to prolonged ischemia is accompanied by formation of oxygen radicals and other oxidants, collectively referred to as “reactive oxygen species” (ROS) (3). This may induce a condition of “oxidative stress reperfusion” damage, in addition to the injury induced by ischemia itself (4,5).

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Subsequently, several reports had pointed out that a variety of antioxidants and scavengers might ameliorate the ultrastructural changes and functional disturbances produced by ischemia-reperfusion in the heart (6). These observations prompted the hypothesis that a better myocardial protection could be achieved with the addition of antioxidants or related substances to the cardioplegic solution during coronary artery by-pass. Further on, we were able to show that supplementation of the cardioplegic solution with mannitol (7) or deferoxamine (8), the preoperative intravenous infusion of taurine (9) and the preoperative administration of vitamins E and A (10) produce a reduction of tissue damage and oxidative stress. We also reported that cardioplegia with blood supplemented with mannitol showed better results than plain crystalloid solutions in patients with normal ventricular mass (11).

Nevertheless, while myocardial ischemia-reperfusion sequence of events has been clearly documented in experimental models (12-17), in cardiac surgery patients, in spite of a wide spectrum of papers (7-9,18-24), evidence in this regard had not been gathered in a systematic, comprehensive fashion, so as to link the various alterations together and to provide an encompassing picture of the phenomenon. This may limit our ability to devise appropriate strategies for optimal perioperative myocardial protection.

We present here a review of our experience in myocardial ischemia-reperfusion in a historical perspective.

3. ISCHEMIA-REPERFUSION. HISTORICAL DATA

All the data from previous studies (2,7-11,25) was grouped and analysed. It consisted of a control ischemia-reperfusion group and five treatment groups (mannitol, deferoxamine, taurine, vitamins A and E, and blood) (7-11,25). Therefore, we grouped 70 patients who underwent elective coronary bypass surgery in order to obtain a highly homogeneous group.

The patients fulfilled the following criteria: (a) ejection fraction greater than 45%; (b) absence of recent (less than 4 weeks) myocardial infarction; (c) absence of associated valve disease; (d) achievement of a complete or satisfactory surgical revascularization. All patients underwent an identical surgical protocol. Briefly, cardiopulmonary bypass was instituted and the temperature of the perfusate was lowered to 28°C. Two full-thickness biopsies were obtained from the apex of the heart before the aorta was cross clamped, and were designated as preischemia samples, one for hydroperoxide-initiated chemiluminescence assay to evaluate oxidative stress (26) and the second for electron microscopy. After placement of the aortic clamp, 500 ml of Saint Thomas-type cardioplegic solution was injected at a temperature of 4°C. After completion of the distal anastomoses, the aortic clamp was removed and the heart defibrillated. Ten minutes after reperfusion, two new biopsies were obtained from

the same area, processed in a similar manner and designated as reperfusion samples.

There was no significant difference in age, ejection fraction, aortic cross clamp time and number of grafts among groups. The treatments were: a) mannitol group (n = 6), the cardioplegic solution contained 59.8 mM mannitol (Laboratory FADA, Bs Aires) (7); b) deferoxamine group (n = 7), the cardioplegic solution was supplemented with 1g/L deferoxamine (Ciba-Geigy, Bs Aires) (8); c) taurine group (n = 6), 1-3 hours before surgery patients received a rapid intravenous infusion of 5 g of taurine (O-due, Nativelle, Florence, Italy;) (9); d) vitamins E and A group (n = 8), patients received an oral administration of 400 mg of vitamin E and 100,000 IU of vitamin A daily for 5 days before surgery (10); and e) blood group (n = 10), blood cardioplegia supplemented with 40 mmol/L mannitol and 20 mmol/L KCl was used (11).

Electron micrographs were taken at $\times 10,000$ and mitochondrial damage was determined in a blind manner by two different observers who assigned a single score to each of the areas (27). A value of 0 through 4 was assigned for each mitochondrion, depending on the degree of morphologic damage, as follows: 0; normal mitochondria; 1; early swelling with separation of cristae and clearing of matrix density; 2; more marked swelling than in grade 1; 3; massive swelling with membrane disruption; and 4; massive swelling with rupture of inner and outer mitochondrial membranes. The average obtained from two blind observers was expressed as a percentage of the total number of mitochondria counted per sample. Approximately 500 mitochondria were graded per biopsy. Mitochondrial damage index was calculated dividing the percentage of damaged mitochondria (grades 3 and 4) in the reperfusion sample by the same percentage of damaged mitochondria in the preischemia sample of the same patient.

All patients had a satisfactory postoperative outcome and no evidence of myocardial infarction was observed. Oxidative stress indexes were determined as the ratio of hydroperoxide-initiated chemiluminescence reperfusion/preischemia in the biopsies obtained from each patient. An oxidative stress index of 2.13, indicated the occurrence of oxidative stress (26) during the reperfusion of the human heart (Table 1) and implies a 113% increased hydroperoxide-initiated chemiluminescence which, is understood as a decreased content of vitamin E and other related antioxidants in the heart biopsy. This decreased content of tissue antioxidants suggested a previous increased rate of oxygen free radical reactions in the heart with the antioxidant consumption (26). Treatments decreased the oxidative stress index by 44% (deferoxamine) to 100% (vitamins E and A) (Table 1). The decreased values of the oxidative stress indexes in the treatment groups are mainly due to a decrease in the after-reperfusion sample; the basal samples of the treatment and control groups were not significantly different (+/-15%).

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Table 1. Oxidative stress in the heart of patients subjected to coronary revascularization surgery

Groups	N	Oxidative Stress Index	Protection (%)
Control	33	2.13 ± 0.18	----
+ mannitol	6	1.06 ± 0.10	94
+ deferoxamine	7	1.63 ± 0.20	44
+ taurine	6	1.18 ± 0.16	84
+ vitamins A and E	8	0.96 ± 0.08	100

The oxidative stress index of 2.13 was taken as 100%, the theoretical value of 1.00 as 0% and the corresponding percentages were correlated therefrom. All treatments, except deferoxamine, were statistically ($p < 0.01$) different from the control ischemia-reperfusion condition. Deferoxamine treatment has a difference with $p = 0.05$.

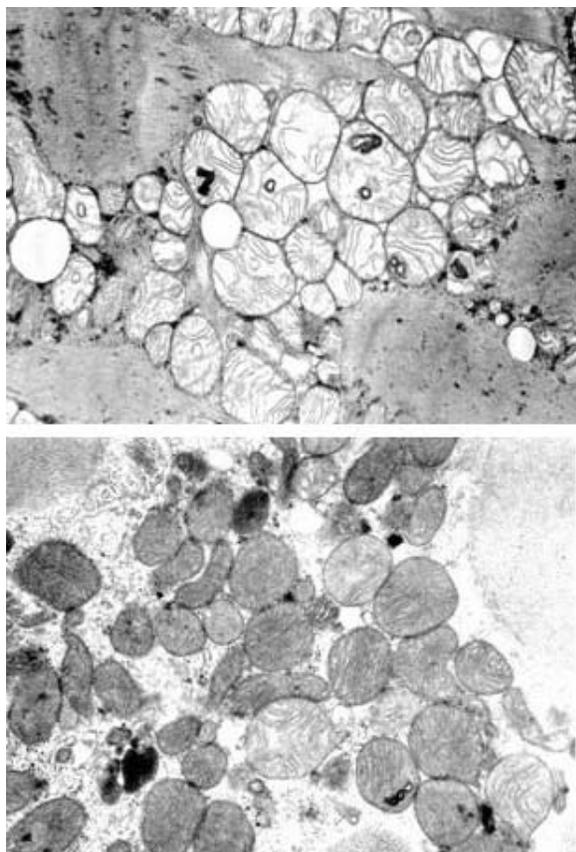


Figure 1. Left panel: Reperfusion of biopsy sample from patient in control group. Damage to mitochondria is severe with massive swelling and disruption of cristae or marked swelling and rupture of membranes. X10,000. Right panel: Reperfusion biopsy sample from patient in taurine group. Mitochondria have intact membranes and tightly packed cristae. X10,000 (From ref. 9).

Basal samples in all the groups showed a normal ultrastructure. The morphology of the reperfusion biopsies of the ischemia-reperfusion group (control) showed focal myofibrillar disorganization with myocytolysis, sarcoplasmic vacuolization and mitochondrial swelling and disruption. Distension of sarcoplasmic reticulum and T tubules were frequently seen and diffusely scattered in damaged myocytes. Mitochondria showing swelling, clearing of matrix density, and separation of cristae with granular and linear densities or myelin figures and

membrane disruption were observed in highly damaged samples (Figure 1) (Table 2).

From the concept of “the oxygen paradox” (28), an extensive research has been produced to clarify its physiopathology. In our experience the use of mannitol (7), deferoxamine (8), taurine (9), vitamins E and A (10), and blood cardioplegia supplemented with mannitol (11) ameliorated reperfusion damage by decreasing oxidative stress and mitochondrial damage.

Experimental studies in rabbits (17,29-31) also supported the occurrence of an oxygen free radical overproduction and oxidative stress in myocardial ischemia-reperfusion.

When we grouped the data of our previous trials (7-11) the indexes of oxidative stress and of mitochondrial damage showed a clear statistical correlation ($r = 0.95$; $p < 0.001$) (Figure 2). The mechanism by which oxygen free-radicals are generated during reperfusion is still under debate but the current hypothesis is that during reperfusion, oxygen encounters a highly reduced mitochondrial respiratory chain that triggers a high production of superoxide anion (O_2^-) at the inner mitochondrial membrane (32). After dismutation of O_2^- at the mitochondrial matrix, the resulting hydrogen peroxide (H_2O_2) diffuses out to the cytosol, reacts with myoglobin, and produces hydroxyl radical (HO) that initiates the free radical chain reaction.

Regarding the substances used by us in order to ameliorate ischemia-reperfusion lesions, in general all share antioxidant properties and its benefits have been widely explained elsewhere (33).

In brief, mannitol has an action as free radical scavenger, particularly referred to HO, and an osmotic effect through the hyperosmotic activity of the extracellular medium (7). Deferoxamine (8) diminishes free iron toxic effects. Vitamins A and E are able to scavenge peroxy radicals (ROO) generated during the propagation phase of lipid peroxidation. In addition, vitamin A effectively quenches singlet oxygen (10). Taurine (9,34) protects against loss of mechanical heart function through a calcium biphasic effect that depends on calcium concentrations (9).

In 1989 a new concept in myocardial protection was initiated as an alternative to the standard use of hypothermia (35). This technique involved continuous,

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Table 2. Mitochondrial damage in the heart of patients subjected to coronary revascularization surgery

Groups	N	Mitochondrial Damage Index	Protection (%)
Control	19	2.63 ± 0.28	----
+ mannitol	6	1.30 ± 0.20	63
+ deferoxamine	7	1.86 ± 0.24	47
+ taurine	6	1.25 ± 0.23	85
+ blood cardioplegia	10	1.08 ± 0.21	95

Mitochondrial damage indexes were determined as the ratio of the percentage of irreversibly damaged mitochondria after reperfusion/before reperfusion in the biopsies obtained from each patient. The mitochondrial damage index of 2.63 was taken as 100%, the theoretical value of 1.00 as 0% and the corresponding percentages were calculated therefrom. All treatments, except deferoxamine, were statistically ($p < 0.01$) different from the control ischemia-reperfusion condition. Deferoxamine treatment was different with a $p < 0.05$.

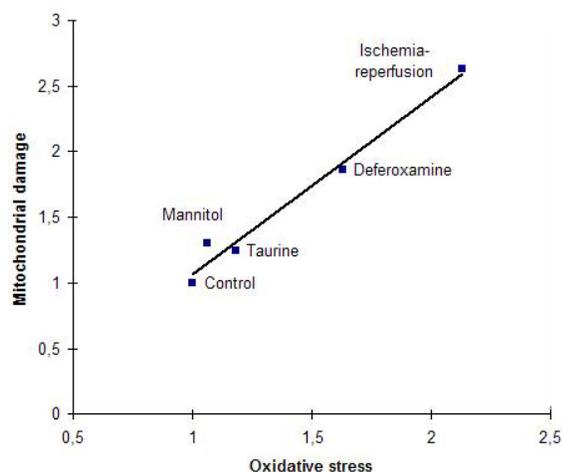


Figure 2. Relationship between the Indexes of Oxidative Stress and of Mitochondrial Damage in heart biopsies of patients subjected to coronary revascularization surgery with different treatments.

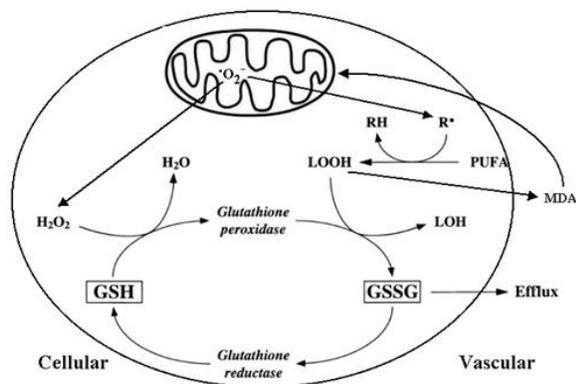


Figure 3. Schematic representation of intracellular inactivation of hydroperoxides in the glutathione cycle. GSH reacts with peroxides undergoing oxidation to GSSG, which is then reduced back to GSH. However, GSSG in excess of glutathione reductase ability leaves the cell via a specific efflux system. H₂O₂, hydrogen peroxide; GSH, reduced glutathione; GSSG, oxidized glutathione; LOOH, lipid hydroperoxide; PUFA, polyunsaturated fatty acid; R[•], free radical; LOH, lipid alcohol. Modified from ref. 16.

normothermic blood-potassium perfusion during the cross clamp period, which aims to provide an uninterrupted supply of oxygenated blood to the myocardium (36). Crystalloid cardioplegic solution might have at least three major disadvantages compared with blood cardioplegic solution: a) it supplies only small quantities of O₂ to the myocardium; b) it substantially increases the hemodilution of the patient, and c) it decreases the oncotic pressure (37,38). Blood cardioplegia appears superior to crystalloid solutions and the benefits of both mannitol and blood cardioplegia were observed in patients with normal ventricular mass in whom an adequate preservation of the ultrastructure in the reperfusion samples was achieved (11).

From the “historical data” we concluded at that time, that although several issues still remained unsolved, there was no doubt that oxygen-free radicals were important contributors to myocardial injury during the reperfusion period of coronary artery bypass surgery. Yet, in spite of this wealth of information, both clinical and experimental, subsequent clinical trials conducted over the last several years with a variety of antioxidant strategies have been largely disappointing (18-24). Therefore, the whole paradigm of oxidative stress in cardiac injury needs to be re-evaluated

4. PERSPECTIVES

How can we reconcile these apparently disparate or even contradictory findings? Several mechanisms may contribute: One possibility is that the magnitude of oxidant-mediated injury in nowadays cardiac surgery is less than anticipated on the basis of previous studies. Several observations lend credence to this hypothesis. First, experimental studies have typically been performed under experimental conditions which generally induced an ischemic insult more severe than what is seen clinically. Second, even with respect to clinical studies, since the ‘80s major advances have been made with respect to cardiopulmonary bypass technique, oxygenator design, cardioplegia, anesthesia, as well as patient’s therapy prior to surgery, all resulting in improved myocardial protection. Third, average aortic cross-clamp time in earlier studies was 55 +/- 5 minutes, whereas at our institution it is now of 40.9 +/- 11.9 minutes. This may be important, since oxidative stress is expected to increase with the length of arrest (39,40). Another potentially important issue has to do with the actual meaning of certain metabolic alterations.

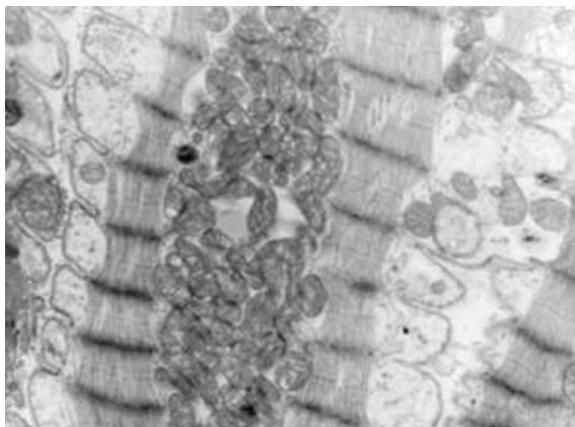


Figure 4. Reperfusion sample. Mitochondria with degree I-II damage are observed, with marked cytosolic edema and glycogen absence. A lipid droplet is seen as well as the sarcolemmal preservation. X8,000

During reperfusion after cardioplegic arrest, trans-cardiac release of glutathione during reperfusion has been observed by previous investigators (39,41), and more recently confirmed by our group (Milei et al., unpublished observations). Nevertheless, even though cardiac formation and release of glutathione is induced by oxidants (42-45), and found during postischemic reperfusion (16,46), this is simply related to ROS exposure, since formation of oxidized glutathione is one of the major intracellular defense mechanisms against oxidants (Figure 3). Only when oxidant load exceeds endogenous detoxification mechanisms oxidant attack progresses to damaging cell constituents. In fact, direct intracoronary infusion of oxidants at concentrations sufficient to elicit glutathione release much larger of what found in this and previous clinical studies, has no detrimental effects on the heart, which nonetheless can be induced by further up-titration of oxidants (43,45,47) (Figure 3). Thus, in and of itself, glutathione release does attest to oxidant attack, but it cannot be taken as proof of oxidant damage. Indeed, in preliminary observations in patients with stable angina scheduled for elective coronary artery bypass surgery, we found that oxidative stress did not progress into oxidant attack to cardiac organelles, nor did it translate into established ultrastructural damage (Milei et al., unpublished observations).

Myocardial ultrastructure was also largely preserved. In particular, alterations that characterize reperfusion injury, namely contraction-band necrosis, massive mitochondrial swelling, sarcolemmal disruption, were largely absent (Figure 4). Accordingly, cardiac function was also preserved (Milei et al., unpublished observations). Those data would suggest that no major oxidative injury occurred in the heart of patients subjected to cardiac surgery under modern approaches.

Another, and perhaps very important factor which may explain the apparent paradox of antioxidant therapy failure, is the occurrence of myocardial "preconditioning", *i.e.* the condition by which the heart becomes more tolerant toward a subsequent insult of ischemia/reperfusion. It has

been shown that exposure to a small amount of oxidants may in fact "precondition" the heart (48). This phenomenon may also occur during surgical procedures, and it is believed to confer additional benefits to isoflurane anesthesia (49,50). It is tempting to speculate that if the actual amount of oxidants formed is modest, there might be more harm than good in trying to eradicate oxidant production. According to this line of thinking, patients receiving antioxidants prior to and/or during cardiac surgery might thus be deprived of this potential benefit (which would make them more tolerant of cardioplegic arrest), while not gaining much from reduction of an otherwise small oxidant load at reperfusion.

In conclusion, patients subjected to elective bypass surgery undergo oxidative stress upon reperfusion after cardioplegic arrest; however, widespread antioxidant therapy must await the result of further studies aimed at characterizing more precisely the true impact of this phenomenon of myocardial cells.

5. ACKNOWLEDGMENT

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