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Oxidative stress: clinical implications in cardiac surgery

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Despite improvements in surgical and myocardial protection techniques, postoperative ventricular dysfunction after cardiac surgery is clinically common and observed experimentally and may represent global stunning . Evidence of a role for oxygen-derived free radicals is overwhelming in most experimental preparations; nevertheless the confirmation of findings in the postischemic human myocardium has been elusive, as we must rely on indirect criteria to assess oxidative stress and the effects of anti-free radical interventions in a clinical setting. None of the studies prove that the magnitude of oxygen-derived free radical formation found is harmful, although they do support the overall concept that oxygen-derived free radicals are formed during reperfusion and may contribute to reperfusion injury. Furthermore, Bolli et al.[1] using electron paramagnetic resonance showed a linear relation between the magnitude of oxygen-derived free radical generation and the magnitude of ischemic flow reduction. These findings imply that whatever may be the precise mechanism responsible for stunning, such a mechanism must be initiated and modulated by ischemia, and any intervention that improves perfusion during ischemia would be expected to attenuate stunning after reflow.

From this point of view we looked with great interest at the method of myocardial protection developed by Lichtenstein et al.[2], the basic concept of which is the association of the chemical electromechanical arrest (by potassium) with a continuous warm blood perfusion to prevent myocardial ischemia. We studied the alterations of glutathione redox status (taken as an indirect index of oxidative stress) occurring in plasma and erythrocytes measured in two groups of 10 patients each undergoing two different techniques of myocardial protection for coronary artery bypass surgery[3]: 1) cold blood cardioplegia (CBC) group: intermittent CBC and warm modified reperfusion in moderate hypothermic (28 C) cardiopulmonary bypass; 2) warm blood cardioplegia (WBC) group: continuous WBC in mild hypothermic (34.5 C) cardiopulmonary bypass. Samples were collected from the coronary sinus and peripheral vein before anesthesia, before aortic unclamping (T2), 15 and 30 min after unclamping (T3, T4). Hemodynamic parameters were obtained with thermodilution technique and related to a possible correlation between oxidative stress and myocardial function in the early postoperative hours. The CBC group showed an increase in oxidized glutathione and glutathione-cysteine mixed disulphide in the coronary sinus plasma, and the overall redox balance of glutathione decreased ($p < 0.01$) at T2, T3, and T4 from basal time and from the WBC group. Comparable results were obtained in the coronary sinus erythrocytes of the CBC group. Furthermore in our study no statistically significant correlation between the duration of ischemia and the appearance of biological signs of oxidative stress was measured in either group. Moreover the most meaningful finding in our series was the non occurrence of correlation between oxidative stress and immediate postoperative ventricular dysfunction; indeed there was no significant difference between groups in common hemodynamic parameters mea-

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sured or need of pharmacological or mechanical support with improved and uneventful post-surgical courses.

We concluded that perhaps oxygen-derived free radicals may play an important role in *in vivo* myocardial reperfusion stress, but endogenous self-defensive antioxidative enzyme systems are also triggered with insignificant myocardial cellular damage as a consequence; indeed the complete reduction of that oxidative stress does not affect the postoperative recovery of myocardial function in a low risk carefully selected group of patients.

More recently we focused our interest on transplantation procedures where, despite overall good results, early patient survival continues to be significantly influenced by preservation, as evidenced by increasing mortality rates as the ischemic time is extended. At present, cold storage is the most common method accepted for myocardial preservation, even if it exhibits a 5-hour limit period. During hypothermic storage ATP rapidly degrades, and this degradation results in the formation of the end products: adenosine, inosine and hypoxanthine. Moreover the prolonged ischemia reduces the naturally occurring defense mechanism of the heart against oxygen-derived free radicals such as mitochondrial superoxide dismutase and glutathione. Considerable research has been focused on maintaining these compounds during ischemia periods and it is recognized as important in monitoring myocardial purine metabolism and antioxidant status during the preservation period in order to evaluate the effects of cardioplegic solutions.

We have developed a high performance capillary electrophoresis method with UV detection (185 nm) for the simultaneous analysis of intracellular free ribonucleotides, nucleosides, bases and glutathione (oxidized and reduced forms) myocardial tissue without thiol derivatization[4]. This simple and fast capillary electrophoresis procedure reveals the complete spectrum of purine metabolites and the reduced/oxidized glutathione ratio found in myocardial extracts. The small sample volumes used in the capillary electrophoresis method make it possible to work with limited biological materials.

We evaluated the effects of Celsior and St. Thomas cardioplegic solutions on the energy and antioxidant cellular state during cardiac transplantation in 10 cases divided into two groups: myocardial perfusion with Celsior solution (Group A) and perfusion with St. Thomas solution (Group B). Celsior solution in contrast to St. Thomas solution contains L-glutamate as metabolic substrate and reduced glutathione as antioxidant. Although the real mechanisms are not clarified, L-glutamate could supply ATP resynthesis. Glutamate is the only amino acid metabolized by the myocardium and during anoxia it is converted to succinate, an intermediate of Krebs cycle. L-glutamate furnished during ischemia and reperfusion provides to succinate depletion preventing mitochondrial unbalance and promote ATP resynthesis. Reduced glutathione in cardioplegic solutions supplies an important antioxidant that is rapidly consumed during the ischemia-reperfusion sequence. Transmural left ventricular biopsies were obtained from the donor heart before explantation procedures (t1), after the cold static preservation (t2), and at 30 min from reperfusion. Specimens were immediately (within 20 s) rinsed of blood in ice-cold, isotonic buffer solution and frozen in liquid nitrogen. The samples were homogenized at 10% with 0.4 M perchloric acid and neutralized. Protein content was determined in the resuspended acid insoluble fraction by the Bio-Rad protein assay and aliquots of the extracts were analyzed by the capillary electrophoresis method for their nucleotide, nucleosides, bases and glutathione content.

Group A showed a higher energy phosphate preservation than Group B during the cold static ischemia period t2 (ATP decreased by 36 and 47% of basal values respectively). The reduced/oxidized glutathione ratio in this situation evidenced no significant variations between the two groups. During reperfusion (t3) differences in the ATP/ADP ratio were even more evident. In Group B these parameters were unchanged compared to t2 while in Group A a significant increase of both ATP/ADP and reduced/oxidized glutathione ratios was evident.

Despite a better ATP and reduced glutathione preservation by the use of the Celsior solution, transplantation procedures however involve an important loss of cellular energetic and antioxidant potential indicating that the possibility of extending the ischemic period is still low.

The chemical reactions involving oxygen-derived free radicals have extremely short kinetics, however we currently lack a simple and reliable technique that would allow direct identification of these species. So far, only electron spin-resonance and spin-trapping have been designed to provide a more direct visualization of oxygen-derived free radicals.

We set up a direct method to determine the occurrence and time course of oxygen-derived free radical production in the coronary sinus blood from patients undergoing heart transplantation[5]. The blood samples collected from the coronary sinus at various times (0, 1, 2, 3, 4, 5, and 10 min) after release of the aortic cross-clamp, were mixed with a 50 mM solution of α-phenyl N-ter butyl nitrone (PBN). Each sample was immediately frozen in liquid N_2 and stored at -80 C. Toluene extracted samples were analyzed by electron paramagnetic resonance. Spectra were recorded at room temperature on a Bruker 200D spectrometer working at X-band and interfaced with Stelar software. Thirty patients were studied. Electron paramagnetic resonance signals were qualitatively similar and differed from each other only in intensity; maximal PBN adduct production occurred at the beginning of blood flow restoration and subsequently declined, approaching the baseline level after 10 min of reperfusion. Our study demonstrates that, during the

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reflow of the transplanted heart, the formation of oxygen-derived free radicals is detectable in the patient s coronary sinus blood using the *ex vivo* spin trapping technique. This technique may be utilized to assess the efficacy of protective procedures in the course of graft implantation.

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