Oxidative Stress in Patients Undergoing Cardiac Surgery: Comparative Study of Revascularization and Valve Replacement Procedures

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Objective. The aim of this study was to evaluate the time course of oxidative stress markers in plasma and erythrocyte from patients undergoing open heart surgery with cardiopulmonary bypass (CPB) and to examine whether the type of surgical technique used (valve replacement or coronary revascularization) produces any differences in these makers.

Patients and methods. Twenty-two patients undergoing cardiac surgery with CPB were divided in 2 groups (valve replacement or coronary revascularization). We took 5 blood samples at different times during cardiac surgery and analyzed thiobarbituric acid reactive substances (TBARS), α-tocopherol, coenzyme Q, and retinol in plasma and TBARS (baseline levels and induced by Fe²⁺-ascorbate oxidation), α-tocopherol, coenzyme Q and catalase, superoxide dismutase, and glutathione peroxidase activity in erythrocyte.

Results. Plasma α-tocopherol content decreased after starting CPB in both groups. In contrast, in erythrocytes there was an increase in the activity or concentration of all of the antioxidants. Erythrocyte TBARS contents, both baseline levels and induced levels, were higher in coronary revascularization group.

Conclusion. Although both groups suffered an increase in oxidative stress after CPB, this increase was higher in coronary revascularization group and therefore the possibility of post-CPB complications could be more severe in this group. As the groups followed a different pattern of antioxidant response, a different therapeutic approach may be required for each.

Key Words: oxidative stress; valve replacement; coronary revascularization; cardiopulmonary bypass; erythrocyte; TBARS; antioxidants.

INTRODUCTION

An ever-increasing number of patients are undergoing cardiac surgery, involving either coronary revascularization or the replacement of heart valve structures (valve replacement) [1, 2]. Both surgical procedures normally require the use of cardiopulmonary bypass (CPB) [1, 2].

It is known that CPB patients are subjected to a high degree of surgical risk [1–4]. In addition to the unphysiological hemodynamic conditions, one complication that may occur during CPB is the ischemia–reperfusion syndrome [3, 5, 6]. During this syndrome, functional, structural and metabolic alterations are caused by a number of factors, one of which is the continuous generation of free radicals arising, among other causes, from the introduction of oxygen during reperfusion [3, 5, 6]. The free radicals produced during CPB give rise to lipid peroxidation, thus damaging the cell membrane [7].

Free radicals are considered a factor responsible for systemic inflammation, one of the most significant aspects of the harmful effects of CPB and which may contribute to the development of several postoperative complications. According to some authors, free radicals are also responsible for causing reperfusion-derived arrhythmias and myocardial alteration [8, 9].

Valve replacement and coronary revascularization patients follow a similar intraoperative procedure [1, 2], so it can be considered that both type of patients are under a similar degree of damage. However, in each
TABLE 1

Patients Clinical Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Revascularization</th>
<th>Valve replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.1 ± 3.4</td>
<td>58.5 ± 3.8</td>
</tr>
<tr>
<td>Associated cardiac pathology?</td>
<td>Yes 21.4%</td>
<td>No 20.0%</td>
</tr>
<tr>
<td></td>
<td>No 78.6%</td>
<td>Yes 80.0%</td>
</tr>
<tr>
<td>Other pathologies</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Haematological illness</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Transfusion before surgery</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Endocrine illness</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Severe or chronic anaemia</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Surgery time (min)</td>
<td>220.5 ± 11.9</td>
<td>210.5 ± 15.4</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>130.0 ± 7.6</td>
<td>128.2 ± 10.5</td>
</tr>
</tbody>
</table>

All of the chemicals products and solvents, of highest grade available, were acquired from Sigma (St. Louis Mo) and Merck (Darmstadt, Germany).

The blood was collected in heparinized tubes and centrifuged at 1750 × g for 10 min at 4°C in a Beckman GS-6R refrigerated centrifuge (Beckman, Fullerton, Calif) to obtain the plasma. The isolation of membranes and cytoplasm from the erythrocyte was performed according to the protocol of Hanahan and Ekholm [11]. The concentration of proteins in the erythrocyte membrane and cytoplasm was determined by the method of Lowry et al. [12].

Coenzyme Q, α-tocopherol and retinol in plasma were determined by High-performance liquid chromatography (HPLC), using the method described by Litarru et al. [13], slightly modified. Previous extraction was carried out using a mixture of hexane, ethanol:isopropanol (95:5) and sodium lauryl sulfate (2%). The hexane phase was removed and dried under a stream of nitrogen. The dry extract was re-suspended in the HPLC mobile phase (ethanol:water, 97:3). The coenzyme Q and the membrane α-tocopherol were also determined by HPLC. These molecules were extracted by a mixture of ethanol:petroleum (60:40), after centrifugation at 2500 g for 5 min, the upper layer was collected by aspiration and the residue was re-extracted twice with 1 ml of petroleum ether. The dry residue of combined extracts was diluted in the HPLC mobile phase (ethanol:water, 97:3). In both techniques the HPLC system consisted of an apparatus equipped with a Diode Array Detector, model 168 (Beckman Instruments, Inc. Fullerton, Calif) and the column was a reverse-phase C18 Spherisorb ODS 1 of 25 × 0.46 cm reverse phase C18 column with a guard column containing the same material as the main column.

Catalase activity was determined following the method described by Aebi [14], based on monitoring the H₂O₂ decomposition, a consequence of the catalytic activity of catalase, by spectrophotometric measures at 240 nm. Superoxide dismutase was determined by the method of Fridovich et al. [15], based on the inhibition by superoxide dismutase of cytochrome C reduction and spectrophotometric measurement at 550 nm. For glutathione peroxidase, we used the technique of Flohé et al. [16], a method based on the instantaneous formation of glutathione oxidized during the reaction catalyzed by glutathione peroxidase.

TBARS were measured spectrophotometrically [17]. The sample was mixed with TBA and acetic acid (pH 3.0) and heated at 90°C for 30 min. To lower the metal-catalyzed auto-oxidation of lipids, BHT (0.01%) was added to the TBA reagent. After cooling, the chromogen was extracted in n-butanol and the absorbance of the organic phase was determined at 535 nm.

In erythrocyte membrane, we performed two separate determinations of TBARS, the first one, referred as baseline levels of TBARS, was made using the erythrocyte with no further induction. The

Anesthetic and Surgical Procedure

All of the patients received scopolamine and midazolam before operation. Noninvasive monitoring was established and, under local anesthesia, the arterial system was cannulated. This procedure enables the internal monitoring of arterial tension and the patient’s metabolic and blood-gas status condition. It was also used to obtain the blood samples (total volume 20 mL) required for this study. Samples were obtained at the following stages of the intervention: the reference level (T0) was obtained immediately after cannulation of the arterial system, before surgical intervention. We then administered hypnotics, opiates and muscle-relaxing agents, performed endotracheal intubation and established mechanical ventilation depending on the respiratory physiopathology of each patient. Anesthesia was maintained with perfusions of fentanyl, atracurio and propofol. After this, the central vein was cannulated and surgery began. The second sample (T1) was taken 10 min after beginning sternotomy. The third sample (T2) was taken after 15 min of cardiopulmonary bypass, and the fourth sample (T3) after 45 min. The final sample (T4) was obtained at 20 min after the removal of the cardiopulmonary bypass.

Analytical Results

All patients, in accordance with the Helsinki agreement, gave their informed consent to the study, which was approved by the Ethics Committee of the “Virgen de las Nieves” Hospital, Granada, Spain.
second determination was developed after the induction of lipoperoxidation by adding Fe$^{2+}$ ascorbate (70 $\mu$M and 280 $\mu$M, respectively) [18].

### Statistical Analysis

Results are presented as mean ± standard error of the mean (SEM). One-way analysis of variance was used to test the time-dependent change in oxidative stress markers. Statistically significant differences ($P < 0.05$) were determined with student’s $T$ test and Bonferroni correction was used in case of multiple comparisons. Statistical processing was carried out using the SPSS package (SPSS for Windows,9.0.1,1999, SPSS Inc., Ill).

### RESULTS

No surgery or anesthesia-related complication arose during this study. Plasma antioxidants (coenzyme Q, $\alpha$-tocopherol, and retinol) are shown in Table 2. In general, a similar pattern was presented for both studied groups. Nevertheless, differences were observed between the two groups, especially with respect to the content of $\alpha$-tocopherol. Thus, patients subjected to coronary revascularization presented higher $\alpha$-tocopherol concentrations with statistically significant difference ($P < 0.05$) at T3 and T4, with respect to those obtained in valve replacement group. With respect to plasma TBARS (Table 2), no significant differences were observed neither between the two groups nor among the samples in each group.

Protein concentration of the erythrocyte membrane (Fig. 1A) and cytosol (Fig. 1B) showed a sharp decrease after starting CPB in both studied groups, with significant differences at T2, T3, and T4 with respect to the reference level (T0). In erythrocyte, all antioxidants, both enzymatic (Fig. 2) and nonenzymatic (Fig. 3), presented in both groups, an increase in activity or concentration after starting CPB. However, there were differences between the 2 groups, as was observed in plasma. Valve replacement patients presented a higher level of activity for all antioxidant enzymes after starting CPB, with statistically significant differences at T0, T1 and T4 for catalase (Fig. 2A), T2 for glutathione peroxidase (Fig. 2B) and T2 and T3 for superoxide dismutase (Fig. 2C) with respect to those in revascularization patients. In contrast, revascularization patients presented higher concentration of $\alpha$-tocopherol (Fig. 3A), with significant differences at T0, T1 and T2. Coenzyme Q (Fig. 3B) did not show significant differences between groups.

Baseline levels TBARS in valve replacement patients’ erythrocytes (Fig. 4A) did not show any significant difference among the times. Conversely, revascularization patients showed a statistically significant increase in TBARS amount (baseline levels) at T2 and T3 with respect to the reference level (T0). After the induction of lipoperoxidation by adding Fe$^{2+}$ ascorbate, TBARS (induced levels; Fig. 4B) followed a similar pattern. However, the observed increase was higher in revascularization patients with statistically significant differences at T2, T3, and T4 with respect to those in valve replacement patients.

### DISCUSSION

In this study, as shown in the clinical characteristics of the patients, the groups are fairly homogeneous in composition and do not present significant differences concerning the surgical procedure in itself. The two groups were subjected to a similar degree of stress, as the intraoperative procedure was comparable [1, 2]. However, it should be noted that these patients presented different pathologies and thus received different medical treatments. This consideration could affect the organism’s response to a similar degree of oxidative stress. We considered it appropriate to maintain the differences in the medical protocols, as the principal objective of this study was to observe the response of 2 groups of patients to a given degree of oxidative stress under the normal conditions in which an operation is conducted without introducing any alterations.

In plasma a similar pattern of behavior was observed
among the patients subjected to valve replacement or to coronary revascularization. Plasma TBARS did not show significant differences neither between the two groups nor among the samples in each group. Toivonen et al. [19], working with patients subjected to cardiac surgery with CPB did no found significant changes in plasma TBARS levels during the cardiac surgery and in other studies, quantification of the content of malondialdehyde in plasma has produced varied results [19].

However, the marked fall in the levels of plasma α-tocopherol in both groups is indicative of the high degree of stress undergone by the patients during CPB.

FIG. 1. Protein concentration in erythrocyte membrane (A) and erythrocyte cytosol (B) for the coronary revascularization and valve replacement patients. Values shown are mean ± SEM. *P < 0.05 compared with reference level (T0) within the same group. †P < 0.05 compared with the corresponding value of the valve replacement group. T0: before surgical intervention; T1: 10 min after sternotomy; T2: 15 min after starting CPB; T3: 45 min after starting CPB; T4: 20 min after finalizing CPB.

FIG. 2. Activity of catalase (A), glutathione peroxidase (B), and superoxide dismutase (C) enzymes in erythrocytes for the coronary revascularization and valve replacement patients. Values shown are mean (SEM). *P < 0.05 compared with reference level (T0) within the same group. †P < 0.05 compared with the corresponding value of the valve replacement group. T0: before surgical intervention; T1: 10 min after sternotomy; T2: 15 min after starting CPB; T3: 45 min after starting CPB; T4: 20 min after finalizing CPB.
The hemodilution accompanying CPB could be in part responsible for remarkable reduction in plasma antioxidative capacity in cardiac surgery patients. In our study we did not use any dilution factor in plasma, because we considered that it is important to reflect an actual amount of both free radical reactions products and plasma antioxidant capacity.

With respect to erythrocyte protein, the results shown a sharp decrease in its concentration after starting CPB in both, erythrocyte membrane and cytosol, which is due to the lost of red cell mass during and immediately after CPB [1, 4].

The antioxidants in erythrocyte, both enzymatic and non enzymatic, presented an increase in activity or concentration after starting CPB. We speculate that in the erythrocyte there is a rapid antioxidant response to the increase in free radical production due to CPB. Some studies in the literature, although working with either different type of cells or different oxidative stress sources, showed that after a short-term oxidative stress induction there was in the cells an increase in the activity of some antioxidant enzymes and this activity decreased to the normal level after a short period of time [22, 23]. It should be noted that the
interaction between the human body and cardiopulmonary bypass apparatus is very complex and involve several oxidative stress sources [1, 2], which may increase the activity of these antioxidant enzymes through allosteric activation for immediate needs.

Concerning the high values of α-tocopherol in plasma and erythrocytes among the coronary revascularization patients, in comparison with those obtained for the valve replacement patients, we believe these to be due to the administration of vitamin E (200 mg every 8 h during the 24 h prior to the operation) to this group of patients as part of the standard medical protocol. Although this treatment could have been omitted, we believed it is correct to maintain the differences between medical protocols to determine the real response of these patients to oxidative stress, and thus be able to evaluate the efficiency of current medical protocols.

Despite the higher α-tocopherol content in the revascularization group, the results seem to indicate a higher degree of oxidative stress in this group (from the TBARS results, both baseline and induced values). This could be the result of the lower rate of increase in the activity of the cytosolic antioxidant enzymes in this group, as it seems increasingly apparent that some endogenous antioxidants, such as glutathione peroxidase, superoxide dismutase, and catalase, act as a primary defense mechanism, whereas others, including vitamin E, seem to play a secondary role in attenuating the damage caused by ischemia-reperfusion [24].

From the limited data available in the present study, it is unclear why these antioxidant enzymes, especially glutathione peroxidase and superoxide dismutase, should present a lower rate of increased activity among the revascularization group, despite presenting similar rates of activity in the basal measurement (T0) with respect to those of the valve replacement group. Further investigation is necessary to clarify this question. Nevertheless, apart from the fact that these patients have a different physiopathology from the valve replacement group, which could alter the allosteric activation mechanisms for the immediate necessities of these antioxidant enzymes, a possible explanation could lie in the above-mentioned administration of vitamin E.

Despite the evidence concerning the protective effect of vitamin E against ischemia-reperfusion obtained from experiments with animals [24], observational and epidemiological data are contradictory [24, 25]. It has been shown that antioxidant supplements (β-carotene and α-tocopherol) can reduce or eliminate the mechanisms of ischemic preconditioning in protecting the myocardium, which would involve an immediate increase in the activity of cytosolic antioxidant enzymes [25, 26]. A similar mechanism might be active in the case of the patients in the present study. Nevertheless, and as remarked above, our data need to be supplemented with further investigation in this field.

In general, our results reveal a sharp increase in oxidative stress after a cardiopulmonary bypass is established, both in plasma and in erythrocytes. This increase in oxidative stress is higher in the coronary revascularization group than in the valvuloplasty group. Although the organism is capable of reducing this stress by means of various antioxidant defenses, this reaction leads to their depletion, increase the susceptibility against oxidative stress, as has been shown after induction of lipoperoxidation by adding Fe²⁺-ascorbate, and therefore an increase possibility of post-CPB complications, which could be more severe in revascularization group.

Despite the limitations arising from the small number of patients selected, the results obtained in this study are significant and the data provided constitute a persuasive argument for a wide-ranging intervention protocol and perhaps for preventive and therapeutic measures, such as the possibility of increasing antioxidant enzyme activity by pharmacological means, both before CPB is established and after it is removed, at least in coronary revascularization patients. The data are also applicable to the preparation of more detailed studies into the generalized use of antioxidants among such patients.

REFERENCES


