Oxidative stress injury after on-pump cardiac surgery: Effects of aortic cross clamp time and type of surgery

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Background: Cardiopulmonary bypass (CPB) in on-pump cardiac surgery can have harmful systemic effects, triggered in part by radical oxygen species (ROS) produced by ischemia-reperfusion in the heart and the lung. We determined the relationship between levels of oxidative stress markers (8-isoprostane and nitrites/nitrates) in plasma with aortic cross clamp duration in patients undergoing cardiac surgery with CPB.

Methods: Thirty patients with CPB were studied: 14 with coronary artery bypass graft surgery and 16 with valve surgery. Plasma levels of 8-isoprostane, and nitrites/nitrates were measured over a 24-hour time course: before (T0) and after CPB: 5 minutes (T1), 1 hour (T2), 12 hours (T3), and 24 hours (T4).

Results: Plasma levels of 8-isoprostane and nitrites/nitrates increased early after CPB, with a subsequent and progressive decline. Levels of oxidative stress markers in T1–T2 were positively correlated with the aortic cross clamp duration. Aortic cross clamp duration times greater than 50 minutes were correlated with higher oxidative stress levels. There were no significant differences in the levels of oxidative stress markers between surgery types.

Conclusion: Cardiac surgery with CPB is associated with an early increase of oxidative stress markers in systemic blood. Aortic cross clamp duration is positively correlated with oxidative stress injury.

Keywords: On-pump cardiac surgery, Cardiopulmonary bypass, Ischemia-reperfusion, Oxidative stress, 8-Isoprostane, Nitrites/nitrates

Introduction

Cardiopulmonary bypass (CPB) is a part of cardiac surgery that has been associated with harmful effects in several organs. These effects are due to a systemic inflammatory response to CPB and ischemia-reperfusion phenomena that affect the myocardium and other organs, such as lungs.^{1–6} Radical oxygen species (ROS) production is a major event involved in myocardial dysfunction related to CPB.⁷ The main sources of ROS are mitochondrial cytochrome oxidase, the arachidonic acid cascade, the xanthine oxidoreductase system, and the NADPH oxidase system in neutrophil granulocytes. Excess ROS reacts with several molecular components (glutathione,

DNA, proteins, membrane phospholipids), leads to post-oxidative cellular modification, and has been implicated in the pathogenesis of post-ischemic contractile dysfunction and dysrhythmias.^{1–8}

The isoprostanes are a family of stable products of arachidonic acid, produced by the random oxidation of tissue phospholipids by ROS without the direct action of the cyclooxygenase enzyme. Increased levels in blood and increased excretion of 8-isoprostane (8-iso-PGF_{2α}) in urine have been found in several pathological situations, and due to its chemical stability, it is considered a reliable index of lipid peroxidation *in vivo*. Urinary levels of 8-isoprostane have been measured previously during coronary reperfusion in patients with on-pump coronary artery bypass graft surgery (CABG).^{9–15}

Nitrites (NO₂⁻) and nitrates (NO₃⁻) are end products of endogenous nitric oxide (NO[•]) and peroxynitrite

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anion (ONOO^-) metabolism. $\text{NO}^\bullet$ and superoxide anion ($\text{O}_2^{\bullet-}$) react to form ONOO^- , which is a powerful oxidant that induces lipid peroxidation, oxidation of arachidonic acid, inactivation of enzymes, and other proteins by oxidation or nitration. ONOO^- is also a potent trigger of DNA strand breakage. Nitric oxide and its metabolites, nitrites and nitrates, have been commonly used to determine nitrosative stress.^{16–19}

The aim of this study was to determine whether there is a direct correlation between the aortic cross clamp duration and oxidative stress marker levels in systemic blood of patients undergoing CPB (CABG surgery and valve surgery). The levels of oxidative stress markers, 8-isoprostane and nitrites/nitrates, were measured over a time course in the systemic blood of patients after leaving the pump. We also determined the effects of type of surgery (CABG or valve surgery) on oxidative stress.

Materials and methods

Patients

Thirty patients undergoing elective on-pump cardiac surgery with CPB were descriptively studied: coronary bypass ($n = 14$), aortic valve ($n = 10$), and mitral valve ($n = 6$). The group with valve surgery (16 patients) included five patients with a valve replacement associated to one or more coronary arterial bypass grafts. The hospital ethical committee approved this study. Informed consent was obtained from all patients. Inclusion criteria were age greater than 18 years and American Society of Anaesthesiologists physical status III or less. Exclusion criteria were previous cardiac surgery, severe hepatic disease, severe renal failure, severe chronic obstructive pulmonary disease, preoperative antioxidant therapy, and pregnancy.

Anaesthetic and surgical management

Anaesthesia was induced intravenously with midazolam (0.1 mg/kg), etomidate (0.3 mg/kg), and fentanyl (2–4 $\mu\text{g/kg}$). Muscle relaxation was obtained with cisatracurium besilate (0.1 mg/kg), with doses repeated when needed. Anaesthesia was maintained with an inhalatory mixture (air, oxygen with FiO_2 0.5 and 2% sevoflurane) in all patients. Patients were ventilated with a volume-controlled mode (tidal volume: 7–8 ml/kg, respiratory rate 12–14 and a positive end-expiratory pressure: 5 cm H_2O) to maintain normoxia and normocapnia.

Cardiopulmonary bypass

After systemic administration of heparin (300 UI/kg body weight), CPB was initiated in a standard manner with cannulas placed in the ascending aorta and right atrium. A non-pulsatile pump and hollow-fiber oxygenators were used. CPB was maintained at

a flow rate of 2.4 l/minute/ m^2 . Moderate hypothermia was used (32–33°C), and local hypothermia for myocardial protection was achieved by antegrade infusion of a cool cardioplegic solution (Cardi-Braun), administered intermittently (every 25–30 minutes average) during clamping. After removing the cross clamp, protamine was given for heparin reversal.

Blood collection

Five arterial blood samples were collected (tubes BD Vacutainer LH PST II with lithium heparin, UK) from a radial artery line before CPB (T0), and at specific time points after CPB: 5 minutes (T1) 1 hour (T2), 12 hours (T3), and 24 hours (T4). The blood samples were centrifuged at 3000 rpm for 12 minutes. Plasma was separated and stored at -80°C until analysis.

Determination of systemic oxidative stress marker levels

The 8-isoprostane was measured using an enzyme-immunoassay method (STAT-8-isoprostane, EIA kit from Cayman Chemical Co., Ann Arbor, MI, USA) with a detection limit of approximately 2.7 pg/ml.

Total nitrites/nitrates were measured by conversion of nitrate to nitrite using nitrate reductase, then the Griess reagent was used to convert nitrite into a compound that was measured colorimetrically (nitrate/nitrite colorimetric assay kit from Cayman Chemical Co.).

Statistical analysis

Results are shown in tables and figures as mean $\pm$ standard deviation (SD). The data were analyzed by one-way analysis of variance. For parametric data, the Student *t*-test was used. For non-parametric data, the Mann–Whitney *U* test and Wilcoxon signed-rank test were used. Pearson's correlation coefficients were determined between different parameters of oxidative stress in blood plasma (8-isoprostane or nitrites/nitrates) and aortic cross clamp time. Also a correlation between the two markers was performed. *P* values <0.05 were considered statistically significant. The Statistical Package for the Social Sciences® (version 11.0) was used for analysis (SPSS, Chicago, IL, USA). Excel (Microsoft Office 2007, Redmond, WA, USA) was used for graphics.

Results

Demographic and preoperative data together with clinical parameters and the intraoperative values of aortic cross-clamp duration (57.1 ± 17.8 minutes), CPB duration (72.7 ± 20.7 minutes), and surgery duration (207 ± 33 minutes) are listed in Table 1.

Table 1 Clinical characteristics and operative data of patients

Characteristics	Group (<i>n</i> = 30)
Age (years)*	67.1 ± 9.5
Sex (M/F)	22/8
BMI (kg/m ²)*	27.9 ± 3.0
Diabetes mellitus (<i>n</i>)	7
Hypertension (<i>n</i>)	6
History of myocardial infarction (<i>n</i>)	5
Cigarette smoking (<i>n</i>)	10
Dyslipemia (<i>n</i>)	16
Statins therapy (<i>n</i>)	16
β-blockers therapy (<i>n</i>)	12
LVEF (%)*	57.4 ± 7.8
CABG (<i>n</i>)	14
Number of grafts per patient*	2.9 ± 0.7
Aortic valve replacement (<i>n</i>)	10
Mitral valve replacement (<i>n</i>)	6
Valve replacement ± CABG (<i>n</i>)	5
Aortic cross-clamp time (minute)*	57.1 ± 17.8
CPB (minute)*	72.7 ± 20.7
Surgery duration (minute)*	207 ± 33

*Values expressed as mean ± SD.

BMI, body mass index; LVEF, left ventricular ejection fraction; CABG, coronary artery bypass graft; CPM, cardiopulmonary bypass.

Fig. 1 summarizes the results and describes the levels of 8-isoprostane and nitrites/nitrates in blood plasma after CPB over the time course (T0–T4). All points (T1–T4) were significantly higher after CPB

(compared to T0), with the initial peaks (T1 and T2) having the greatest significance ($P < 0.001$). The levels progressively decreased in T3 and T4, but remained statistically significant: T3 ($P < 0.01$), T4 ($P < 0.01$, only in nitrites/nitrates). The correlation performed between 8-isoprostane and nitrites/nitrates (Fig. 1C) showed at T2 a moderate but significant value ($R = 0.5270$; $P = 0.0028$). Although, at T1 the correlation was very weak ($R = 0.2311$; $P = 0.2192$).

We found a positive correlation between the aortic cross-clamp times and the blood plasma levels of 8-isoprostane and nitrites/nitrates in the times T1 and T2. Correlations in T1 were moderate for 8-isoprostane: $R = 0.503$ ($P < 0.01$), and weak for nitrites/nitrates: $R = 0.364$ ($P < 0.05$). Values were weak in T2 for both markers: $R = 0.391$ ($P < 0.05$) and $R = 0.480$ ($P < 0.01$), respectively, but all were statistically significant (Fig. 2).

As a reference, we took the median value of the aortic cross-clamp duration (50 minutes) from all patients ($n = 30$), then formed two groups of 15 patients each: one with cross-clamp durations of less than 50 minutes (<50 minutes), and the other with durations greater than 50 minutes (>50 minutes). We found a significant increase of 8-

Figure 1 Time course levels of 8-isoprostane (8-iso-PGF_{2α}) in plasma (A). Time course levels of nitrites + nitrates (NO₃⁻ + NO₂⁻) in plasma (B). Pearson's correlation coefficients (*R*) were determined between the parameters of oxidative stress (8-isoprostane and nitrites + nitrates) in blood plasma (C). Five arterial blood samples were collected: previously to CPB (T0), 5 minutes after CPB (T1), 1 hour after CPB (T2), 12 hours after CPB (T3), 24 hours after CPB (T4). Values expressed as mean ± SD (*n* = 30). Differences in the same group (T0 vs. T1–T4) were analysed with a one-way analysis of variance and two-tailed Student *t*-test. *P* values <0.05 were considered statistically significant. Statistical differences were observed in the same group: ***P* < 0.01; ****P* < 0.001.

Figure 2 Pearson's correlation coefficients (R) were determined between different parameters of oxidative stress in blood plasma (8-isoprostane and nitrites + nitrates) and aortic cross clamp time (min). Blood plasma levels of 8-isoprostane (A and B) and nitrites + nitrates (C and D) in the times T1 (A and C) and T2 (B and D) were moderately or weakly correlated with the aortic cross clamp duration (minute), but statistically significant in A, B, C and D. P values <0.05 were considered statistically significant. Five arterial blood samples were collected: previously to CPB (T0), 5 minutes after CPB (T1), 1 hour after CPB (T2), 12 hours after CPB (T3), and 24 hours after CPB (T4).

isoprostane and nitrites/nitrates at each time point after CPB (T1–T4), with the greatest increases in the early time points (T1 and T2) (Fig. 3). We also

found significant differences in oxidative stress marker levels in T1 and T2 between the two groups (<50 minutes vs. >50 minutes), with higher levels

Figure 3 The mean of aortic clamp duration was 50 minutes. We formed two groups of patients: 15 patients with an aortic cross clamp time <50 minutes; and 15 patients with an aortic cross clamp time >50 minutes. We observed a significant increase of oxidative stress plasma levels in the time course (T0 vs. T1–T4) of 8-isoprostane (8-iso-PGF_{2α}) (A) and nitrites + nitrates (NO₃⁻ + NO₂⁻) (B). Statistical differences were observed in the time course of each group (T0 vs. T1–T4), in aortic cross clamp time <50 minutes (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$), and in aortic cross clamp time >50 minutes († $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$). Furthermore, we find differences between the two groups (aortic cross clamp time <50 minutes vs. aortic cross clamp time >50 minutes) at T1 and T2 in 8-isoprostane levels (A) and, at T2 in nitrites + nitrates levels (B). Values expressed as mean $\pm$ SD. Differences were analyzed with a one-way analysis of variance and two-tailed tests for non-parametric data: Mann–Whitney U and Wilcoxon tests. P values <0.05 were considered statistically significant. Five arterial blood samples were collected: previously to CPB (T0), 5 minutes after CPB (T1), 1 hour after CPB (T2), 12 hours after CPB (T3), 24 hours after CPB (T4).

Figure 4 We have divided all patients ($n = 30$) on two groups: a group with patients undergoing coronary surgery ($n = 14$), and other group with patients undergoing valve surgery ($n = 16$; this group included five patients with a valve replacement $\pm$ CABG). We observed a significant increase of oxidative stress plasma levels in the time course (T0 vs. T1–T4) of 8-isoprostane (8-iso-PGF_{2 α}) (A) and nitrites + nitrates (NO₃⁻ + NO₂⁻) (B). Statistical differences were observed in the time course of each group (T0 vs. T1–T4): in valve surgery (** $P < 0.01$; *** $P < 0.001$) and, in coronary surgery († $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$). However, we did not find differences between these two groups. Values expressed as mean $\pm$ SD. Differences were analyzed with a one-way analysis of variance and two-tailed tests for non-parametric data: Mann–Whitney U and Wilcoxon tests. P values < 0.05 were considered statistically significant. Five arterial blood samples were collected: previously to CPB (T0), 5 minutes after CPB (T1), 1 hour after CPB (T2), 12 hours after CPB (T3), and 24 hours after CPB (T4).

in the group with the longer aortic cross-clamp duration (> 50 minutes), particularly in 8-isoprostane values (see Fig. 3).

We also studied the effect of the type of cardiac surgery on the oxidative stress marker levels. We separated all patients ($n = 30$) into two groups: patients undergoing coronary surgery ($n = 14$), and patients undergoing valve surgery ($n = 16$), although this group included five patients with a valve replacement associated to one or more coronary arterial bypass grafts. We found a significant increase of 8-isoprostane and nitrites/nitrates levels at all time points (T1–T4) after CPB in both groups (Fig. 4). However, we did not find any significant differences between these two groups.

Discussion

Previous studies in patients have demonstrated that cardiac surgery with CPB is associated with ischemia-reperfusion consecutive to cross-clamping.^{2–5,7,8,11} These patients may suffer systemic oxidative stress with an increase of multiple oxidative stress markers and a decrease in antioxidant reserves that can result in increased postoperative morbidity and prolonged hospital stay.^{20–22} Perioperative metabolic therapy or antioxidant therapy improves redox status and outcomes in cardiac surgery patients.^{20–22} Some studies have even shown that the oxidative injury produced by cardiac surgery with on-pump coronary bypass graft surgery is greater than off-pump coronary bypass surgery.^{11,23} Possibly due to the handling of the myocardium during the ischemia period (manipulation time) and after completion of all grafts.²⁴

Our study investigated the time course of the levels of two, well-characterized oxidative stress markers in plasma from patients undergoing elective on-pump cardiac surgery with CPB. We found an early increase of 8-isoprostane and nitrites/nitrates in systemic blood, indicating increased lipoperoxidation and peroxynitrites formation, respectively. Both of their levels initially peaked after CPB, and then began a progressive decrease. These results are in agreement with previous studies,^{2,5,8,11,21,23,25} although the majority of those studies did not measure the levels of 8-isoprostane in blood. However, some of these studies measured the levels of 8-isoprostane in urine. Some studies have measured other oxidative stress markers, such as TBARS, malondialdehyde, oxidized glutathione, protein carbonyls. As far as we can determine, this is the first report where nitrosative stress biomarkers were measured in blood after CPB. Our findings suggest that formation of ROS occurs in the myocardium within the first 5 minutes of reperfusion, consistent with previous reports.^{2,5,8,23} This indicates that there is an early washout of 8-isoprostane and nitrites/nitrates from the reperfused tissues after CPB. Although the correlation between the levels of 8-isoprostane and nitrites/nitrates at T2 (1 hour after CPB) is better than at T1 (just 5 minutes after CPB), possibly by a greater dispersion of values markers in T1.

At the T1 and T2 time points, we found a positive correlation between aortic cross-clamp duration and blood plasma levels of 8-isoprostane and nitrites/nitrates, although with moderate and weak, but significant, values. These data suggest that depending on the severity of the ischemic period, oxidative injury occurs during reperfusion, an effect that could be related to a

delay in postoperative recovery and cardiac dysfunction. It has also been reported that oxidative stress after declamping is inversely related to the recovery of cardiac index.⁵ The mechanism of peroxynitrite-mediated cardiomyocyte injury involves multiple pathways, including nitration and inhibition of cardiac myofibrillar proteins.^{26,27}

After splitting the patients into two groups based on the median value (50 minutes) of the aortic cross-clamp duration, we calculated a significant increase in the two cited biomarkers at all time points compared to T0 for both groups, especially at T1 and T2. There were also differences in T1 and T2 between the two groups, especially in 8-isoprostane levels, with significantly higher levels of oxidative damage over greater cross-clamp duration. These results show that oxidative damage occurs within a short time frame (<50 minutes), although at lower levels than the damage that occurs over longer time frames (>50 minutes). These results indicate that it is important to shorten the time of ischemia to prevent the oxidative injury.

We found a significant increase of 8-isoprostane and nitrites/nitrates levels at all times (T1–T4) in the two types of surgery (valve and coronary). For the first time, we compared the effect of valve surgery versus coronary surgery on oxidative stress marker levels, but we did not find any significant differences between them. However, we did find a trend towards higher levels of oxidative stress markers in patients with valve surgery than in patients with coronary surgery. This trend may be due to greater aggression on the myocardium tissue during valve surgery.

In conclusion, we believe that the results of this study contribute to a better understanding of the pathophysiology of reperfusion of the human heart. This study suggests that considerable oxidative stress occurs early during conventional cardiac surgery with CPB. The degree of oxidative stress in the myocardium and in other tissues depends on the duration of the ischemic period, but does not depend on the type of cardiac surgery. Circulating ROS seems to be involved in secondary myocardial and pulmonary injury. New strategies need to be proven to reduce the systemic oxidative stress and to improve patient outcomes after CPB, which will require large-multicenter studies.

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