Isoprostanes and Oxidative Stress in Off-Pump and On-Pump Coronary Bypass Surgery

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Background. **Conventional on-pump coronary artery bypass grafting (CABG) is associated with a systemic inflammatory response and by an increased production of reactive oxygen species, whereas off-pump coronary artery bypass grafting (OPCAB) is thought to be accompanied by less oxidative stress. Urinary isoprostane iPF$_{2\alpha}$-III is a new marker reflecting oxidative stress; it has emerged as the most reliable marker of oxidative stress status in vivo. This study was designed to ascertain whether OPCAB compared with CABG represents a surgical strategy that avoids oxidative stress. To this end urinary isoprostanes and other established oxidative stress markers were measured during the first 24 hours after CABG and OPCAB.**

Methods. **Fifty low-risk coronary patients were randomly assigned to CABG or OPCAB. Urinary isoprostane iPF$_{2\alpha}$-III levels, plasma levels of free malondialdehyde, and total antioxidant status were measured before, during, and up to 24 hours after surgery.**

Results. **In OPCAB iPF$_{2\alpha}$-III excretion remained unchanged throughout the study. As expected, in CABG iPF$_{2\alpha}$-III levels significantly increased during surgery and returned at baseline 24 hours later. Free malondialdehyde behaved similarly, with no change in OPCAB and sharp increases during CABG. Conversely, total antioxidant status showed a sharp drop during CABG, followed by a slow recovery, whereas a significantly lower drop occurred in OPCAB.**

Conclusions. **In this randomized study in low-risk coronary patients, OPCAB revealed less perioperative oxidative stress, as reflected by lack of excretion of iPF$_{2\alpha}$-III in urine, by lack of increase of plasma free malondialdehyde, and by lower decreases in plasma total antioxidant status.**

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Conventional on-pump coronary artery bypass grafting (CABG) has been previously shown [1–5] to be associated with prothrombotic and proinflammatory response. During this type of surgical intervention reactive oxygen species (ROS) are also produced, together with neutrophil sequestration and activation within vasculature, endothelial damage, and increased vascular permeability. An increase in ROS can overwhelm local antioxidant defense mechanisms and cause damage to biological molecules; eg, DNA, lipids, and proteins. Altogether these effects may represent important contributory factors to the incidence of perioperative or postoperative complications occurring after CABG [6].

The avoidance of cardiopulmonary bypass (CPB), as well as of heart and lung ischemia-reperfusion, has been proposed to reduce the postoperative systemic inflammatory response in off-pump coronary artery bypass grafting (OPCAB). In fact, OPCAB is associated with a somehow lower degree of systemic inflammatory and oxidative response than conventional CABG during the intraoperative period and the very early hours after surgery [1, 5, 7, 8].

The F_2 isoprostanes are a family of free radical catalyzed prostaglandin F_2 isomers that are formed in situ from the fatty acid backbone esterified in membrane phospholipids. They are released in response to cellular activation and have been detected in human plasma and urine [9, 10]. Increased excretion of F_2 isoprostanes in urine has been found in association with advanced age [11], chronic obstructive pulmonary disease [12], cigarette smoking [13], and hypercholesterolemia [14]. Due to their mechanism of formation, specific structural features, and chemical stability, they are considered a reliable index of oxidant stress and ensuing lipid peroxidation in vivo [15]. An increase in the urinary excretion of F_2 isoprostanes has been previously reported in CABG [16], but we have found no information on isoprostane excretion available in patients undergoing OPCAB.

The present study tests the hypothesis that the increases in oxidative markers occurring during CABG are prevented in patients undergoing OPCAB. To this end we assessed the in vivo secretion of urinary excretion of F_2 isoprostanes and the levels in plasma of some established markers of oxidative stress in patients undergoing

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CABG or OPCAB. In addition, the potential correlation of urinary excretion of F_2 isoprostanes with some markers of oxidative stress in plasma was also evaluated.

Patients and Methods

Fifty first-time isolated low-risk (EuroSCORE $<$ 6) coronary bypass surgery patients, for whom an on-pump and an off-pump procedure was deemed technically feasible and not contraindicated (ie, porcelain aorta for CABG), were enrolled in accordance with a protocol approved by the Institutional Review Board (approved January 11, 2002) at the Centro Cardiologico Monzino, and written informed consent was obtained. Exclusion criteria were Q-wave myocardial infarction in the last 6 weeks, unstable angina, or poor left ventricular function (ejection fraction [EF]$<$ 0.30), whereas there were no restrictions for age.

Patients were randomly assigned to CABG (n = 25) or OPCAB (n = 25). The same surgical and anesthetic team managed all patients. None of the patients were taking vitamins, dietary supplements, or drugs with established antioxidant properties during the study. Conventional therapy was allowed according to clinical judgment.

After internal mammary harvesting, heparin (300 IU/kg) was given and activated clotting time was kept 440 seconds or greater with additional heparin in both groups. Upon completion of distal and proximal coronary anastomoses, heparin was antagonized with protamine sulfate at a 1:1 ratio.

CABG

A nonpulsatile roller pump and hollow-fiber oxygenators were used. Cardiopulmonary bypass was initiated with cannulas placed in the ascending aorta and right atrium (two-stage venous cannula). Each operation was performed with tepid hypothermia (32–33°C) and hemodilution. During CPB, blood flow was maintained at 2.4 $L \cdot min \cdot m^2$. Myocardial protection was achieved by the administration of cool, antegrade and retrograde multidose blood cardioplegia.

OPCAB

This procedure was performed by a midline sternotomy with the same heparinization protocol as described above. Mechanical stability of the coronary arteriotomy area was achieved with the Octopus IV system (Medtronic Inc, Minneapolis, MN) and a soft plastic coronary flow-shunt was always passed into the coronary arteriotomy to reduce myocardial ischemia and to improve visualization of the anastomosis field. The hemodynamic management of patients during distal coronary anastomosis consisted mainly in the careful and progressive elevation of the heart with tissue slices, associated with substantial volume administration to allow the heart to adapt to the new positioning and to avoid major hemodynamic derangements or need of inotropic drug administration.

Urine Sampling

An overnight urine collection the night before (pre), 4 to 6 hours after surgery start (during), and an overnight urine collection 24 hours after surgery (after) was carried out. The antioxidant 4-hydroxy-tempo (1 mmol/L; Sigma Chemical Co, St Louis, MO) was added to urine and samples stored at -80°C until analyzed.

Blood Sampling

Blood samples were collected at 7 time-points, from a catheter positioned in the radial artery, in tubes containing ethylenediaminetetraacetic acid (9.3 mM; Vacutainer Systems, Becton Dickinson, Rutherford, NY) as anticoagulant: before induction of anesthesia (t0, pre), after sternotomy (t1), 30 minutes after aortic cross-clamp in CABG and 30 minutes after the start of the first distal anastomosis in OPCAB (t2), after protamine administration (t3), at the end of surgery (t4, about 1.5 hours from t2), 4 hours after the arrival to the intensive care unit (t5), and 24 hours after surgery (t6). After blood centrifugation, plasma was removed and stored at –80°C until analyzed.

Isoprostane Determination

Urinary isoprostane $iPF_{2\alpha}$-III was purified using a double extraction protocol followed by quantification using an enzyme-immunoassay (Cayman Chemical Co, Ann Arbor, MI – SPI-BIO, Saclay, France), according to Wang and colleagues [11], with modification, as previously described [17]. A 20,000 dpm of [^{3}H]-$PGF_{2\alpha}$ was added as internal standard to 2 mL of urine and results were corrected for the recovery and expressed as picogram per milligram of creatinine. Only free (not esterified in phospholipids) isoprostanes can be detected.

Assay of Free Plasma Malondialdehyde (MDA)

Free plasma MDA levels were assessed, after organic extraction, with gas chromatography-mass spectrometry (GC-MS) as previously described [18]. Synthesized dideuterated-MDA was added as internal standard. The GC-MS analyses were carried out on a Hewlett-Packard 5890 gas chromatograph (HP Company, Palo Alto, CA) equipped with an HP-5 fuse silica capillary column (25 m, 0.32 mm id, 0.25 mm film thickness) and coupled to a 5988A mass spectrometer.

Assay of Individual Antioxidant Status (IAS)

Plasma IAS was measured by a commercially available spectrophotometric assay (OXY-Adsorbent Test, Diacron International, Grosseto, Italy). Plasma samples were submitted to massive oxidative stress with hypochlorous acid and IAS values determined by reading the absorbance at 505 nm.

Statistical Analysis

Normally distributed data are reported as means $\pm$ 1 standard deviation; data that were not normally distributed are reported as median and interquartile range (IQR) between brackets. Clinical variables of the patients were compared with two sample t-tests, χ^2, or Fisher's

Table 1. Main Characteristics of the Patients

	CABG (n = 25)	OPCAB (n = 22)	*p* Value
Age	63.4 ± 11.5	68.4 ± 8.9	0.11
Males (%)	17 (68)	18 (82)	0.33
Hypertension (%)	16 (64)	12 (55)	0.72
Diabetes (%)	7 (28)	5 (23)	0.94
Previous acute myocardial infarction (%)	12 (48)	8 (36)	0.61
Chronic obstructive pulmonary disease (%)	1 (5)	2 (8)	>0.99
Chronic renal failure (%)	3 (14)	0 (0)	0.24
Ejection fraction (%)	56.2 ± 13.4	55.8 ± 9.6	0.91
Distal anastomoses	3.1 ± 0.67	2.5 ± 0.67	0.005

CABG = coronary artery bypass grafting; OPCAB = off-pump coronary artery bypass.

exact tests when indicated. Values represented in time course charts are the mean differences (delta ± standard error of the mean) between each time point value and that measured at baseline.

In order to adjust for potential confounding factors (ie, age, gender, EF, previous myocardial infarction, and number of bypass performed), general linear model analysis of covariance models were used for statistical analysis of time, group (CABG vs OPCAB), and interaction (time*group) effects in oxidative stress variables; in this case skewed variables ($iPF_{2\alpha}$-III) were log-transformed before analysis. When time, group, or interaction effects were significant ($p < 0.05$), repeated measures ANCOVA with Bonferroni correction was used to establish significant ($p < 0.05$) point-by-point differences.

To compute within subjects' correlations we first subtracted individual patient means from all values. The resulting residuals were then analyzed by Spearman correlation. All analyses were performed by SAS statistical package v.8 (SAS Institute Inc, Cary, NC).

Results

Clinical Characteristics and Surgical Data

Forty-seven out of the 50 randomized patients successfully completed the study. Three, all in the OPCAB group were excluded for the following reasons: refusal to undergo surgery after randomization (1 patient), conversion to CABG (1 patient), and perioperative myocardial infarction (1 patient). There were no significant differences between groups both in preoperative and intraoperative variables, except for the mean number of grafts implanted (Table 1); all patients who completed the protocol had a normal postoperative course.

Urine Isoprostane Excretion, Plasma MDA, and Total Antioxidant Status

Before surgical intervention, isoprostane excretion of $iPF_{2\alpha}$-III in urine was 98.5 (67.5–155.5 IQR) and 99.5 (56-128 IQR) pmol/mmol creatinine in patients undergoing OPCAB and CABG, respectively (Table 2). In patients undergoing OPCAB urinary $iPF_{2\alpha}$-III, excretion did not change significantly during the study; on the other hand, a consistent increase in isoprostane excretion was detected in CABG patients during surgery (153 pmol/mmol creatinine, 112-257 IQR, $p < 0.01$ vs presurgery), levels returning to baseline 24 hours after surgery (Fig 1A, and Table 2). Similarly, no change in plasma free MDA levels were observed in OPCAB patients, whereas in CABG patients free MDA was significantly higher than baseline and than OPCAB patients from the time point 30 minutes after aortic cross-clamp application (t2) up to surgery end (t4), and peaking after protamine administration (t3) (Fig 1B, and Table 2). Total MDA levels in plasma behaved as free MDA (data not shown).

Interestingly, a statistically significant reduction of IAS occurred both in CABG and OPCAB starting from the time point 30 minutes after aortic cross-clamp application (t2) and lasting up to 24 hours after surgery, the decrease being more marked in CABG at all time points. The IAS levels tended to return to baseline 24 hours after surgery but the recovery was incomplete both in CABG and in OPCAB (Fig 1C and Table 2). After adjustment for age, gender, EF, previous myocardial infarction, and number of bypass performed, data remained substantially unchanged for the three variables ($iPF_{2\alpha}$-III, free MDA, IAS) considered.

In CABG patients a positive ($r = 0.48$, $p < 0.0001$) correlation was found between the changes in urinary $iPF_{2\alpha}$-III and plasma free MDA. In addition, changes in $iPF_{2\alpha}$-III and in plasma free MDA negatively correlated with changes in IAS ($r = -0.53$, $p < 0.0001$ and $r = -0.46$, $p < 0.0001$, respectively).

Comment

This study shows that off-pump coronary surgery markedly reduces the occurrence of an in vivo prooxidant state; the lack of changes in the levels of $iPF_{2\alpha}$-III in urine and of free MDA in plasma, together with an only modest reduction of plasma total antioxidant status in OPCAB, suggests that the contribution of general surgical trauma to the oxidative stress is minimal and affects only the endogenous antioxidant defense mechanism without sensibly increasing the production of prooxidant species.

Altered levels of immunoreactive $iPF_{2\alpha}$-III in urine were reported in the clinical setting of coronary reperfusion [15] and in a variety of syndromes putatively associated with oxidant stress in vivo [16]. Moreover, isoprostanes are known to have biological effects in vitro through membrane receptors for prostanoids [19, 20] and may therefore be considered themselves as mediators of oxidative stress.

Our study does not answer the question of which cells or tissues, subjected to oxidant stress, contribute to the increased isoprostanes excretion and MDA formation detected during surgery in CABG patients. One likely source might be blood cells stressed when circulating through the heart-lung machine. Indeed, activation of granulocytes and subsequent release of oxygen-derived free radicals and granular enzymes are well-recognized

Table 2. Measured Oxidative Stress Markers Over Time

Urine							
					Main Effects (ANCOVA)		
Variable	Group	Pre	During	After	Time	Treatment	Interaction
Urinary $iPF_{2\alpha}$-III (pmol/mmol of creatinine)	CABG	99.5 (56–128)	153 (112–257)[a,b]	101.5 (74–169.5)	0.0148	0.0002	0.0187
	OPCAB	98.5 (67.5–155.5)	101.5 (69–161)	89.5 (60–143)			

Plasma											
									Main Effects (ANCOVA)		
Variable	Group	t0	t1	t2	t3	t4	t5	t6	Time	Treatment	Interaction
Free MDA (μM)	CABG	0.83 ± 0.417	0.80 ± 0.585	2.2 ± 1.48[a,b]	4.2 ± 1.87[a,b]	3.5 ± 2.09[a,b]	1.2 ± 0.82	0.71 ± 0.539	<0.0001	<0.0001	<0.0001
	OPCAB	1.0 ± 0.55	0.70 ± 0.471	0.87 ± 0.876	0.94 ± 0.672	0.83 ± 0.395	0.68 ± 0.323	0.65 ± 0.276			
IAS (μM HCIO/mL sample)	CABG	285 ± 49.9	247 ± 44.4	167 ± 37.0[a,b]	161 ± 41.2[a,b]	178 ± 47.2[a,c]	185 ± 37.4[a,b]	199 ± 33.8[a,c]	0.0167	0.0095	0.69
	OPCAB	280 ± 36.8	240 ± 40.6	220 ± 44.5[a]	195 ± 43.7[a]	204 ± 42.5[a]	226 ± 59.3[a]	226 ± 65.0[a]			

[a] = Significant difference ($p < 0.01$, repeated measures ANCOVA) within each group as compared with baseline; [b] = Significant difference ($p < 0.01$, repeated measures ANCOVA) between CABG and OPCAB groups; [c] = Significant difference ($p < 0.05$, repeated measures ANCOVA) between CABG and OPCAB groups.

ANCOVA = analysis of covariance; CABG = coronary artery bypass grafting; HCIO = hypochlorous acid; IAS = individual antioxidant status; MDA = malondialdehyde.

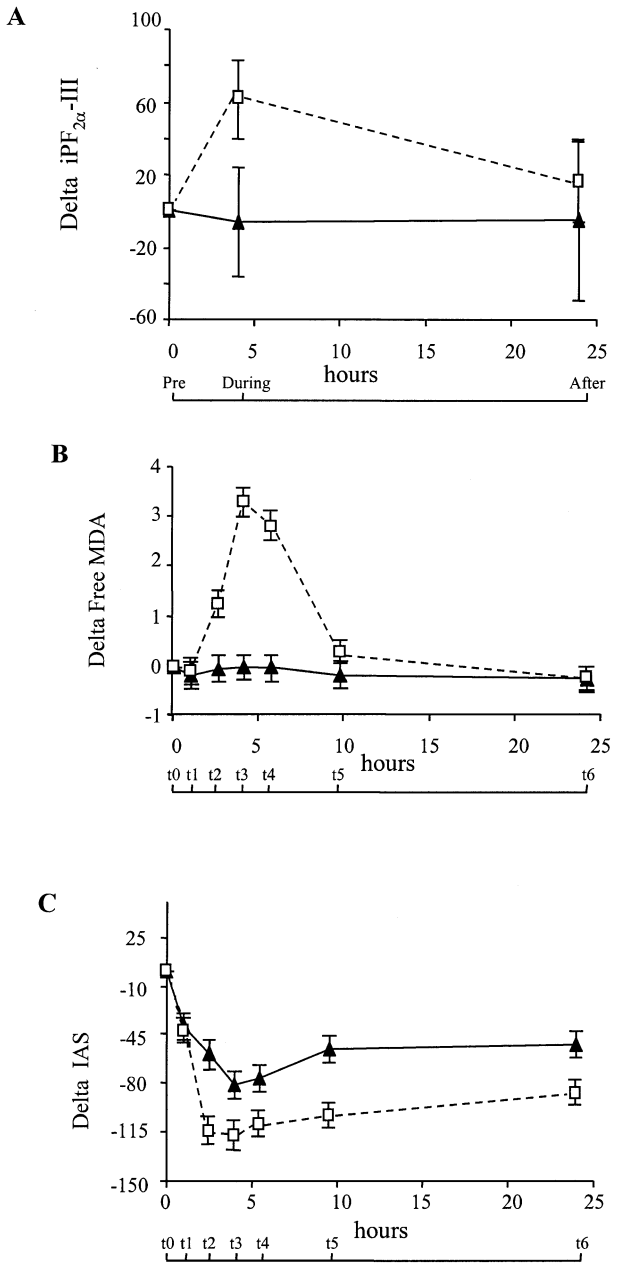

Fig 1. Perioperative difference (delta) in urinary $iPF_{2\alpha}$-III excretion (A), plasma free malondialdehyde (MDA) levels (B), and plasma total individual antioxidant status (IAS) (C). Values represented in time course charts are the differences (delta ± standard error of the mean) between each time point value and that measured at baseline. (□ = coronary artery bypass grafting; ▲ = off-pump coronary artery bypass.)

during cardiac surgery with the use of CPB. Possible sites of leukocyte activation might be the coagulation and fibrinolytic cascades as well as complement activation, likely through the alternative pathway (eg, through blood-circuit contact) [3, 21]. Activated platelets may represent a direct source of isoprostanes as these cells might be significantly triggered by contact activation with the material surface of the heart-lung machine and are able to synthesize isoprostanes in a cyclooxygenase-dependent fashion [22]. Nonetheless, activated platelets may generate isoprostanes and thromboxane B2 in a molar ratio of approximately 1:1000 and it therefore seems difficult to link the increased concentrations of urinary $iPF_{2\alpha}$-III simply with CPB-related platelet activation.

The sharp drop in IAS status detected in CABG patients may reflect an augmented utilization of plasma antioxidants, whereas the increases in $iPF_{2\alpha}$-III and MDA suggest an enhanced oxidative stress. Thus, in CABG patients, free radical generation outweighs the endogenous defense mechanisms and may contribute to global myocardial reperfusion injury [23–25], whereas in OPCAB patients only a reduction in endogenous defense mechanisms was observed, as reflected by a relatively modest, but significant, reduction of IAS.

Thus, OPCAB minimizes intraoperative oxidative stress. Whether or not this protection is enough to prevent short-term or long-term clinical events is not addressed in this trial and requires further investigation. Up to the present time, multiple clinical studies have examined the issue whether or not off-pump coronary grafting is associated with lower mortality and morbidity rates without gaining a consensus [26–28]. In fact, no evidence coming from large, randomized controlled trials is available on these issues and all the available information comes mostly from observational, uncontrolled, nonrandomized clinical trials, which makes their generalization difficult [29].

In conclusion, our results indicate that patients undergoing OPCAB had only mild signs of oxidative stress compared with patients submitted to CABG. Further studies are needed to investigate whether this improved protection against oxidation will result in improved clinical outcomes.

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