

Systemic Free Radical Activation Is a Major Event Involved In Myocardial Oxidative Stress Related to Cardiopulmonary Bypass

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Background: Cardiopulmonary bypass (CPB) can induce deleterious effects that could be triggered in part by radical oxygen species; however, their involvement in the course of surgery has been elusive. The aim of this study was to evaluate the time course and origin of radical oxygen species release, myocardial or not, in patients undergoing coronary artery surgery involving CPB.

Methods: Blood samples were taken from periphery and coronary sinus of patients during CPB, and oxidative stress was evaluated by direct and indirect approaches. Direct detection of alkyl and alkoxy radicals was assessed by electron spin resonance spectroscopy associated with the spin-trapping technique using α -phenyl-*N*-tert-butyl nitron.

Results: The authors showed that the spin adduct concentration was not influenced by anesthesia and pre-CPB surgery. A rapid systemic increase of plasma spin adduct concentration occurred after starting CPB, and it stayed at a high concentration until the end of CPB. At the beginning of reperfusion period, radical oxygen species release was accelerated in the coronary sinus; however, it was not significant. A positive correlation was found between α -phenyl-*N*-tert-butyl nitron adduct concentrations and (1) the duration of CPB and (2) concentration of postoperative creatine phosphokinase of muscle band (CPK MB). Plasma vitamin E and C, ascorbyl radical, uric acid, thiol, plasma antioxidant status, and thiobarbituric acid reacting substances were also measured but did not give relevant indications, except for uric acid, which seemed to be consumed by the heart during reperfusion.

Conclusion: The results indicate that a systemic production of free radicals occurs during CPB that may overwhelm the production related to reperfusion of the ischemic heart. This systemic oxidative stress is likely to participate in secondary myocardial damage.

CARDIOPULMONARY bypass (CPB), a necessary and integral part of cardiac surgery, can itself induce deleterious effects, resulting in diffuse damage of several tissues. Although CPB is routinely performed without sig-

nificant sequela, some patients can develop organ dysfunction involving kidneys, liver, lungs, central nervous system, or cardiovascular system. Technical improvements achieved over the past years have contributed to the reduction of operative and postoperative mortality and morbidity. However, enlarging patterns of surgical indications has concomitantly increased the number of high-risk patients who are more prone to the development of postoperative organ dysfunction.

The pathogenesis of these dysfunctions is multifactorial. It is believed to be triggered in part by a systemic inflammatory response to CPB, induced by the exposure of blood elements to nonphysiologic surfaces.¹ As for heart damage, it could be more specifically associated with myocardial ischemia and reperfusion consecutive to cross clamping and clinically expressed as arrhythmia or "myocardial stunning," a depressed contractile function of major importance in the early postoperative period.²⁻⁴ Evidence suggests that reactive oxygen species (ROS) may play a significant role in the pathogenesis of both of these aforementioned phenomena. A systemic increase of various markers of oxidative stress⁵⁻⁷ has been demonstrated to occur during CPB. Generation of oxygen free radicals could be the result of the activation of neutrophils occurring in response to an inflammatory reaction.¹ Moreover, it is known that ROS generation takes place during myocardial ischemia and reperfusion in various experimental models⁴ and in the human heart.⁸ ROS could therefore be responsible for bypass-induced damages or impairment of myocardial recovery.⁸⁻¹⁰

Despite the extensive research on ROS over the past years, the origin and the exact mechanism of their production in the setting of cardiac surgery remain unclear. Indeed, their chemical nature makes them extremely reactive and short-lived, and renders their detection and quantification very difficult. Observation of free radical production can be achieved by electron spin resonance (ESR) spectroscopy. This technique, which allows the identification and quantification of different free radical species, has been applied in human blood.¹¹

The aim of the current study was to investigate the time course and the origin of ROS release in patients undergoing open heart surgery. For this purpose, peripheral and coronary sinus (CS) time-collected samples were taken during coronary artery bypass grafting, and the oxidative stress was examined by both direct and indirect approaches.

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Table 1. Clinical Status and Operative Data of Patients Included in the First Study

Age (yr)	61 ± 11
Sex (M/F)	8/3
Nicotine addiction	7
Hypertension	3
Hyperlipidemia	7
Diabetes	2
Obesity	3
No. of grafts	
Two	5
Three	4
Four	2
LVEF (%)	62 ± 9
Cross-clamping time (min)	83 ± 31
CPB duration (min)	120 ± 37
Cardioplegia (l)	1.3 ± 0.4
Defibrillation	
Spontaneous	4
Electric shock	7
Weaning	
Simple	8
Amines	3

Plus-minus values are mean ± SD.

LVEF = left ventricular ejection fraction; CPB = cardiopulmonary bypass.

Materials and Methods

Operative Data and Sample Collection

Group 1. We studied 11 patients who were scheduled to have nonurgent aortocoronary bypass grafting. The study protocol was approved and controlled by the local medical ethics committee (CCPRB, Dijon, France). Informed consent was obtained in each case. The same surgeon performed the operation in all patients. Patient characteristics and clinical data are summarized in table 1.

After premedication with hydroxyzine, anesthesia was performed with midazolam, fentanyl, pancuronium, and isoflurane. Ventilation was controlled with oxygen (30%). After systemic application of heparin (300 UI/kg body weight), CPB was instituted in a standard fashion. A nonpulsatile pump was primed with Ringer-lactate solution (1 l) and Eloes solution (0.5 l). CPB was maintained at a flow rate of $2 \text{ l} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$. Moderate body hypothermia (32°C) was used. An anterograde cardioplegia catheter was inserted in the ascending aorta, and a retrograde cardioplegia cannula was placed in the CS. After applying the aortic cross clamp, topical slush was applied on the heart, and 500 ml of St. Thomas Hospital Cardioplegic Solution was infused. Infusions were repeated every 30 min of clamping, resulting in 1–2 l of total cardioplegia. Rewarming began 10 min before removing of the aortic cross clamp, and 100 ml of mannitol solution (20%) was perfused systemically 2 min before reperfusion. Reperfusion on CPB was continued for 25–40 min after removing the cross clamp. Finally, CPB was discontinued, and protamine was given for heparin reversal.

Blood was collected simultaneously from CS and periphery (P) on dry tubes. CS venous blood samples were

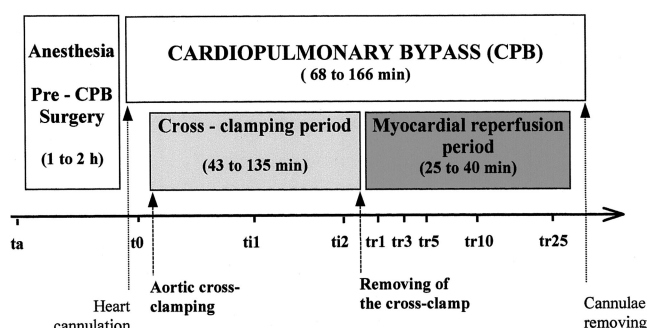


Fig. 1. Schematic diagram of surgery sequences and samples time collection. ta (group 2 only) = preanesthesia sample; t0 = control sample taken just before the beginning of CPB; t1 = 30 min after applying the cross clamp; t2 = just before removing the cross clamp; tr1, tr3, tr5, tr10, tr25 = during the myocardial reperfusion period at 1, 3, 5, 10, and 25 min after removing the cross clamp.

obtained from CS catheter, and arterial periphery samples were taken from the CPB pump (except for the control peripheral blood, which was taken from the pulmonary artery). Schematic diagram of time sample collection is depicted in figure 1. Periphery and CS control samples (t0) were taken before CPB, just after placement of the CS catheter. Two periphery samples were taken during the cross clamping period: t1 30 min after applying the aortic cross clamp and t2 just before removing it. Periphery and CS samples were taken during the myocardial reperfusion period at 1, 3, 5, 10, and 25 min after removing the cross clamp (tr1, tr3, tr5, tr10, and tr25).

Group 2. Another group of 12 patients undergoing cardiac surgery using CPB was included. Anesthesia and surgery were conducted as previously described. Data were collected (fig. 1) before anesthesia, when the patient had just been brought into the operating room (ta), and just before starting CPB, *i.e.*, after the anesthesia procedure and the pre-CPB surgery (t0). In this group of patients, only spin-trapping experiments were performed.

Electron Spin Resonance Spectroscopy Measurements

All ESR spectra were recorded on a Bruker (Wissembourg, France) ESP 300E-X band spectrometer. Plasma samples for ascorbyl free radical (AFR) measurements were introduced into an aqueous ESR quartz flat cell, and ESR spectra were recorded at room temperature using a TM₁₁₀ cavity.^{10,12} Relative radical concentrations (in arbitrary units) were determined by the measurement of spectra line intensities. AFR was expressed as arbitrary units and as arbitrary units per milligram per liter of vitamin C. Erythrocyte suspension was used to determine nitrosylhemoglobin by ESR spectroscopy. ESR spectra were recorded at 100 K in a TM double cavity.¹³ The nitrosylhemoglobin contents were determined from ESR signal intensity and expressed in arbitrary units.

Spin-trapping experiments used α -phenyl-*N*-*tert*-butylnitron (PBN), obtained from Aldrich Chemical Co. (St. Quentin, France). PBN-saline solutions (120 mM) were prepared just before use (10 min stirring, N₂ gassed). Blood samples (5 ml) were immediately mixed with 2.5 ml PBN-saline solution and then centrifuged at 3500 rpm for 5 min. The resultant plasma-spin-trap samples were extracted immediately with 1 ml of toluene (20 s stirring, 10 min centrifugation at 4,500 rpm). The toluene extracts were stored in liquid N₂ for less than 24 h and then transferred into a quartz ESR flat cell for analysis. ESR spectra were recorded at room temperature using a TM₁₁₀ cavity. PBN spin-trap preparation, extractions, storage, and analysis were performed sheltered from light to prevent any photolytic degradation from the trap. The following parameters were used for detection of PBN adducts in toluene extracts: microwave power = 2 mW, microwave frequency = 9.5 GHz, modulation amplitude = 1.6 G, modulation frequency = 100 kHz, scan time = 83.88 s, scan range = 80 G. The concentrations of PBN adducts (nanomolars) were determined through a double integration of the spectra using tetramethylpiperidiny-1-oxyl (TEMPO) nitroxide radical as a standard.

Metabolic Measurements

Protein plasma concentrations (expressed as grams per liter) were evaluated by the spectrophotometric method according to Lowry *et al.*¹⁴ Concentrations obtained in plasma were corrected for hemodilution with regard to the plasma protein concentration by the following equation (except for vitamin E, which was corrected against the cholesterol concentration, and plasma antioxidant status [PAS], which is a dynamic measurement that reflects the antioxidant status of plasma and does not need to be corrected):

$$\text{Corrected value} = \text{measured value} \times (C_{\text{sample}}/C_{t0})$$

where C_{t0} indicates the mean of peripheral and CS plasma protein concentrations before CPB, and C_{sample} indicates the protein concentration of the sample.

Total PAS was determined by the method first described by Cao *et al.*,¹⁵ based on the property of allophycocyanin (Mikralgen, France) to lose its fluorescence on oxygen radical damage.¹² In this assay system, allophycocyanin (37.5 nm) was used as an indicator protein, 2,2'-azobis(2-amidinopropane) dihydrochloride (3 mM) was used as a peroxy radical generator, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma, France) was used as a control standard. The allophycocyanin fluorescence was measured after addition of plasma (1/1,000) at the emission wavelength of 652 nm after excitation at 598 nm every minute at 37°C until disappearance of allophycocyanin fluorescence. Results were expressed as oxygen radical absorbing capac-

ity (ORAC) units, where 1 ORAC unit equals the net protection provided by 1 μ M Trolox.

Plasma total vitamin C (ascorbic acid and dehydroascorbic acid) was measured spectrophotometrically according to the method described by Roe and Kuether¹⁶ and expressed as milligrams per liter. Plasma vitamin E (α -tocopherol) concentrations were quantified by high-performance liquid chromatography assay.¹⁷ Heptan-isopropanol (99/1) was used as a mobile phase, and vitamin E was detected by fluorescence at 295 nm after excitation at 325 nm. Vitamin E concentrations were expressed as micromoles per millimoles of total cholesterol. Total plasma cholesterol concentration was determined by an enzymatic method (Sigma Diagnostics, St. Quentin, France).

Total thiol groups measurements in plasma (micromolars) were assayed according to the spectrophotometric method of Tietze¹⁸ using the Ellman reagent. Uric acid concentrations (milligrams per liter) were determined by the Sigma enzymatic procedure (Sigma Diagnostics) using uricase and peroxidase.

Thiobarbituric acid reacting substance plasma concentrations, expressed as nanomoles per milliliter, were assayed according to a fluorimetric method.¹⁹ Plasma was treated with a reagent containing thiobarbituric acid, and the fluorescence intensity of the complex was measured at 553 nm after excitation at 515 nm. Nitric oxide stable-end metabolites (nitrite and nitrates) plasma concentrations were measured *via* a procedure previously described,¹³ based on the Greiss reaction.²⁰ Total stable-end nitric oxide oxidation products were expressed as micromolars.

Statistical Analysis

All data are presented as mean \pm SD. A Student *t* test was used to compare preanesthesia and pre-CPB values (group 2). Data for group 1 were compared by a one-way analysis of variance (Systat[®] 5.03 for Windows; SPSS Sciences), followed, if appropriate, by a one-sided Dunnett pairwise comparison between control samples (t_0) and others time points. Dunnett pairwise comparison was also used to compare simultaneous CS and peripheral samples. Correlations were evaluated by the Pearson test. A *P* value < 0.05 was considered significant.

Results

Baseline Measurements

Clinical characteristics and operative data of patients from group 1 are shown in table 1. Mean age was 61 \pm 11 yr. All patients had a good left ventricular function as judged by the preoperative angiogram (left ventricular ejection fraction between 53 and 72%) and had no evidence of pulmonary disease. Two to four grafts were performed in each patient, with a mean cross clamping

Table 2. Plasma Concentrations of Some Metabolic Parameters during CPB in Peripheral (P) and Coronary Sinus Blood (CS) of Patients (Group 1)

	Before CPB (t0)		End of Cross Clamping (ti2)		During Myocardial Reperfusion (tr10)	
	P	CS	P	CS	P	CS
PO ₂ (mmHg)	304 ± 95		298 ± 54		341 ± 125	
Pco ₂ (mmHg)	32.3 ± 3.4		32.9 ± 3.5		31.3 ± 2.8	
Proteins (g/l)	62 ± 6	66 ± 9	42 ± 4*		43 ± 10*	42 ± 9*
Vitamin C (mg/l)	9.4 ± 5.2	9.1 ± 5.4	10.8 ± 6.7		9.7 ± 4.8	10.0 ± 4.4
Vitamin E-cholesterol (μmol/mmol)	6.6 ± 1.5	6.4 ± 1.0	6.3 ± 1.1		6.6 ± 1.3	6.4 ± 1.6
NOx (μM)	66 ± 43	60 ± 31	90 ± 58		86 ± 47	92 ± 46
TBARS (μM)	2.1 ± 0.6	2.1 ± 0.5	2.0 ± 0.3		1.9 ± 1.1	2.0 ± 0.8
AFR (AU)	6.4 ± 1.4	6.7 ± 2.6	9.1 ± 2.5		8.6 ± 1.9	9.6 ± 5.0
AFR-vitamin C (AU · mg ⁻¹ · l ⁻¹)	1.04 ± 0.73	0.94 ± 0.54	0.85 ± 0.28		1.03 ± 0.50	0.98 ± 0.48

Values are mean ± SD.

* Significantly different from t0 ($P < 0.001$).

CPB = cardiopulmonary bypass; PO₂ = oxygen tension; Pco₂ = carbon dioxide tension; NOx = nitrites + nitrates; TBARS = thiobarbituric acid reactive substances; AU = arbitrary units; AFR = ascorbyl free radical.

time of 83 ± 31 min and a mean CPB duration of 120 ± 37 min (mean ± SD). Six patients had an electric shock for defibrillation, and two needed amines for weaning. The 11 patients had a satisfactory postoperative outcome.

The second group included 12 patients (6 men and 6 women) with a mean age of 77 ± 6 yr. The time between the preanesthesia and the pre-CPB samples was approximately 1–2 h.

Electron Spin Resonance Studies

An ESR doublet spectrum corresponding to AFR¹⁰ ($g = 2.015$ and $a_H = 1.8$ G) was detected in all plasma samples. The baseline ESR doublet intensity was identical in CS and peripheral samples, and no significant modification of AFR intensity was observed during CPB in CS or peripheral samples (table 2). Similarly, AFR values adjusted to vitamin C did not show any variation during CPB. No nitrosylhemoglobin ESR signal¹³ was detected in the erythrocyte suspensions collected at any time of the CPB period.

Representative ESR spectra of spin adduct extracts obtained from blood of the same patient before and

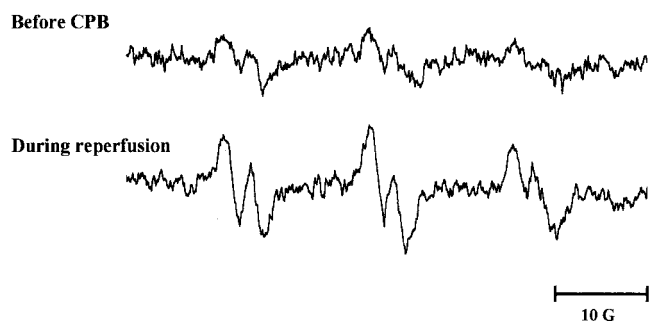


Fig. 2. Electron spin resonance spectra corresponding to alkyl-alkoxy α -phenyl-*N*-*tert*-butylnitron adducts in plasma toluene extracts (see acquisition parameters in Material and Methods) obtained in the coronary sinus of the same patient before cardiopulmonary bypass (CPB) and after 3 min of reperfusion (cross clamping time = 69 min).

during CPB are depicted in figure 2. The ESR spectra detected in all samples ($g = 2.012$, $a_N = 13.5$ G, and $a_H = 2.1$ G) were characteristic of alkyl and alkoxy PBN adducts.¹¹ The PBN adduct plasma concentration did not differ significantly between preanesthesia and pre-CPB samples (group 2), as shown in figure 3 (10 ± 3 nM vs. 13 ± 4 nM). Time course of corrected PBN adduct concentration during CPB (group 1) is shown in figure 4. Before cross clamping (t0), a small amount of PBN alkyl-alkoxy adducts were detected both in CS and in peripheral blood samples at similar concentrations: 10 ± 2 nM and 11 ± 2 nM, respectively. During cross clamping, the spin adduct concentration steadily increased in the peripheral blood, reaching 37 ± 28 nM at ti2 versus 11 ± 2 nM at t0 ($P < 0.01$). During the reperfusion period, spin adduct concentrations stayed high (25 ± 8 nM in periphery at tr25). In the CS, the spin adduct production

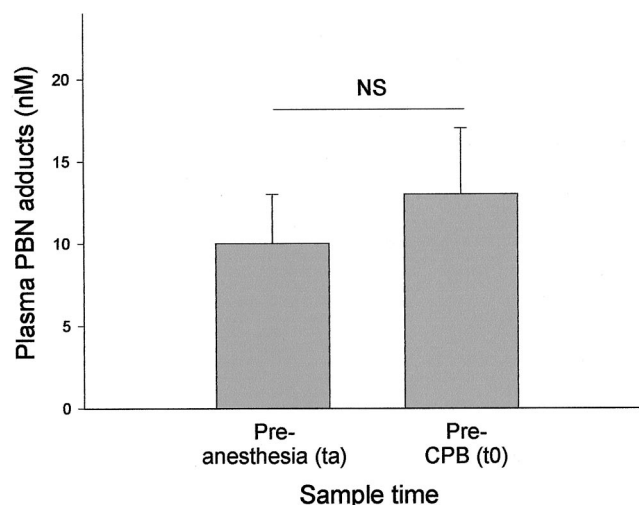


Fig. 3. α -Phenyl-*N*-*tert*-butylnitron (PBN) adduct concentration (mean ± SD) measured in arterial blood of patients before (ta) and after (t0) anesthesia and pre-cardiopulmonary bypass (CPB) surgery (group 2). NS = nonsignificant.

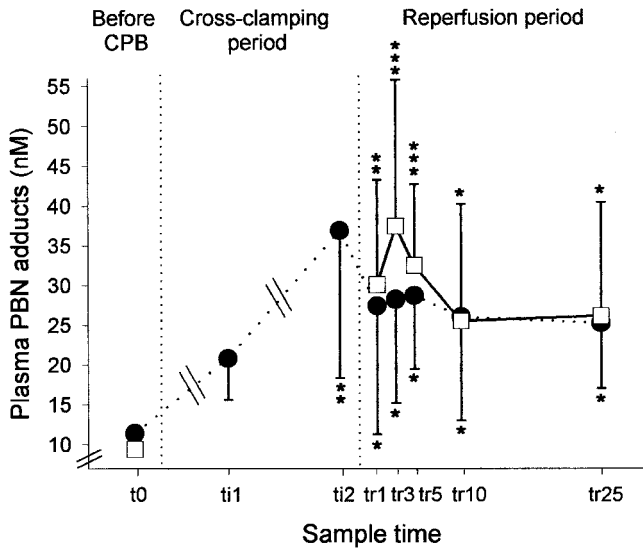


Fig. 4. Time course of α -phenyl-*N*-tert-butyl nitro (PBN) adduct concentration (mean \pm SD) in peripheral (circles) and coronary sinus (squares) blood of patients undergoing cardiopulmonary bypass (CPB; group 1). Significantly different from t0: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

seemed to be more significant during the beginning of reoxygenation: a peak occurred 3 min after declamping in CS samples (10 ± 2 nM at t0 vs. 37 ± 18 nM at tr3; $P < 0.001$). However, no significant difference was found between peripheral and CS samples at this time. Finally, the spin adduct concentration did not recover preclamping values after 25 min of reperfusion, neither in CS nor in peripheral samples. In addition, a correlation existed between the spin adduct production during CPB and the cross clamping duration ($r = 0.81$; $P < 0.01$), and between the spin adduct production and postoperative CPK MB concentrations of the patients ($r = 0.77$; $P < 0.01$).

Metabolic Results (Group 1)

Protein plasma concentration was decreased during the CPB procedure (table 2) identically in the CS and peripheral blood, reflecting hemodilution. Therefore, our results were presented as corrected values, except PAS, which is a dynamic measurement that reflects the antioxidant status of plasma and does not need to be corrected. All measurements taken before the start of CPB (t0) gave equivalent results for peripheral and CS samples.

After 30 min of cross clamping, a significant decrease of the PAS was observed in peripheral and CS samples (1.92 ± 0.55 ORAC at t0 vs. 1.33 ± 0.31 ORAC at ti1 in peripheral samples; $P < 0.05$), which persisted during the entire reperfusion period (fig. 5). No significant modification of the vitamin C plasma concentration was observed throughout CPB (table 2). As shown in table 2, vitamin E values adjusted to cholesterol did not show any variation during the intervention. There was no

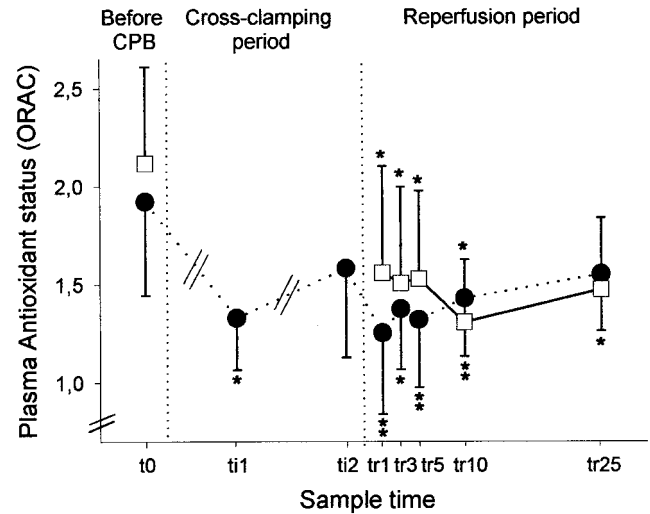


Fig. 5. Plasma antioxidant status expressed as oxygen radical absorbing capacity (ORAC) of peripheral (circles) and coronary sinus (squares) blood of patients during cardiopulmonary bypass (CPB; mean \pm SD). Significantly different from t0: * $P < 0.05$, ** $P < 0.01$.

difference between peripheral and CS vitamin E plasma concentrations at any time point.

As depicted in figure 6, an increase in total thiol concentration was observed as a function of time, with significant differences arising during the reperfusion period in a similar manner in peripheral and CS samples compared with pre-CPB values (453 ± 99 μ M at t0 vs. 620 ± 158 μ M at tr1 in peripheral samples; $P < 0.05$). Peripheral values of plasma uric acid also showed a significant increase during CPB (fig. 7) from the 30th minute of cross clamping (52 ± 9 mg/l at t0 vs. 66 ± 10 mg/l at ti1 in peripheral samples; $P < 0.05$) to the end of the reperfusion period. In CS, the uric acid plasma concentration increased

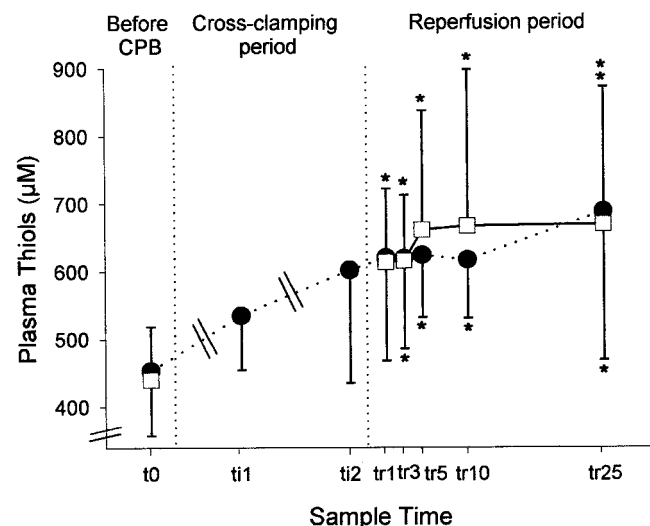


Fig. 6. Plasma concentration of thiols in peripheral (circles) and coronary sinus (squares) blood of patients undergoing cardiopulmonary bypass (CPB; mean \pm SD). Significantly different from t0: * $P < 0.05$, ** $P < 0.01$.

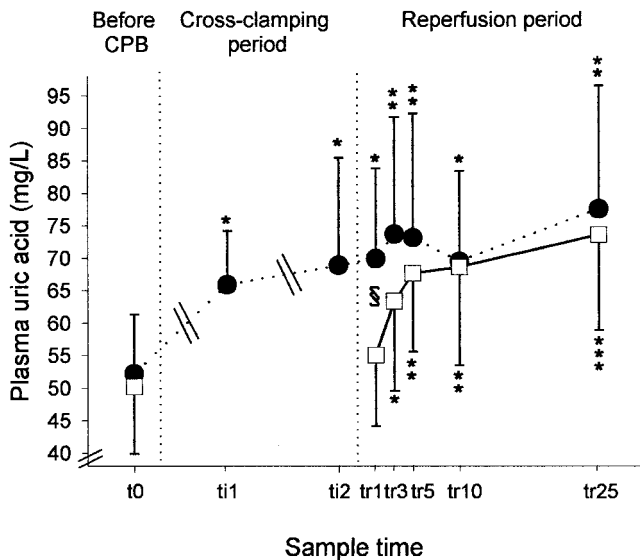


Fig. 7. Plasma concentration of uric acid in peripheral (circles) and coronary sinus (squares) blood of patients undergoing cardiopulmonary bypass (CPB; mean \pm SD). Significantly different from t0: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; §coronary sinus significantly different from peripheral samples at the same time ($P < 0.01$).

progressively after declamping to become similar to peripheral values at 10 min of reperfusion, a significant difference existing between CS and peripheral samples at the beginning of reperfusion (at tr1: 70 ± 16 mg/l in peripheral samples *vs.* 55 ± 12 mg/l in CS; $P < 0.05$).

Thiobarbituric acid reacting substance concentrations did not show any significant evolution during the CPB procedure (table 2). No difference was observed between CS and peripheral thiobarbituric acid reacting substances plasma concentrations at any time point. As reported in table 2, no variation of the stable-end nitric oxide oxidation products was observed during CPB.

Discussion

Our study investigated the evaluation of oxidative stress and the identification of free radicals produced during surgical procedures involving CPB. Our results demonstrate that a systemic oxidative stress occurs in patients undergoing open heart surgery, illustrated by the increased alkyl and alkoxy radicals detected and quantified by ESR spectroscopy.

Many studies have already taken an interest in classical indirect radical stress markers such as vitamins, antioxidant plasma status, or thiobarbituric acid reacting substances^{6,21,22} during cardiac surgery, but the results were controversial. These discrepancies in the results could be attributable to differences in the analytical techniques, operational proceedings, management during CPB, to the variability of patient population, to the large plasma distribution of these compounds, and also

to the expression of the results, corrected or not for hemodilution.

Measurement of free radicals in patients is difficult because of the transient nature of these species. It can be achieved directly by ESR spectroscopy for relatively stable radicals such as plasma AFR and nitrosylhemoglobin; however, their analysis in our study did not show any noticeable modification during CPB. AFR signal is highly sensible to oxygen concentration in the sample analyzed by ESR, and high oxygen tension used during CPB may have interfered with the detection of this radical. The production of nitric oxide has been investigated indirectly in other studies during CPB and gave controversial results, showing either an augmentation,²³ a reduction,²⁴ or, as we showed, no detectable effect of CPB on nitric oxide metabolism.²⁵ Therefore, the potentially beneficial or detrimental functions of this free radical during cardiac surgery remain unclear.

Because of the very short lifetimes of ROS, the spin-trapping technique associated with ESR is an effective approach to examining oxidative stress. In this technique, the unstable radical reacts with a nitron spin trap to produce a more stable nitroxide spin adduct. In our study, the splitting constants of the toluene extract ESR signals suggest that the free radical species trapped by PBN are consistent with either carbon- or oxygen-centered secondary species.¹¹ The presence of these alkyl-alkoxy radicals could result from the action of primary ROS such as hydroxyl or superoxide on cellular and plasma components. On the other hand, these radical secondary species participate in radical chain reactions, in particular lipoperoxidation, and can induce deleterious effects within tissues.^{9,26,27} The increased PBN adduct concentrations in our study support the evidence that an effective free radical aggression occurs during CPB. In addition, our results show that peripheral alkyl-alkoxy radical release exists from the onset (during the cross clamping period) to the end of the CPB procedure independently, at least to some extent, of reperfusion of the ischemic myocardium.

Our findings suggest that a myocardial radical activation seems to occur within the first 3 min of reperfusion in the CS, which is in agreement with previous investigations.¹¹ However, our study established that no significant difference existed between peripheral and CS samples. Therefore, it seems reasonable to hypothesize that the myocardial ROS release could have been masked by the high systemic radical activation preceding the myocardial blood passage. We demonstrated in the second group of patients that no difference occurred in ROS concentration between samples collected before anesthesia or just before CPB, *i.e.*, after the anesthesia procedure and the pre-CPB surgery, which includes the sternotomy, collection of internal mammary artery, or saphenous vein grafts. Therefore, anesthesia and surgical trauma are not likely to be responsible for the peripheral

oxidative stress observed in our study. Other components that could participate in the peripheral free radical production include the poor perfusion of peripheral tissues or the high oxygen tension level used during CPB. Overall inflammation and activated neutrophils could be a prominent source of ROS, as discussed further. On the other hand, the dominant peripheral oxidative stress that we showed does not exclude the contribution of myocardium in the production of free radicals, which is a well-known phenomenon, even if it did not reach significance in our demonstration. It should be noted that it is possibly relevant that mannitol administered just before the myocardial reperfusion could have influenced the level of free radical production detected in the CS. On the other hand, production of primary ROS within myocardium during reperfusion could induce specific intracellular effects that cannot be evidenced in plasma using our spin-trapping technique.

Finally, the positive correlation showed between the rate of PBN adducts produced during CPB and the duration of aortic cross clamping might be attributable to the cross clamping, but also to the duration of CPB itself. Actually, the production of PBN adducts, systemic for a majority, could be the result of the enlarged duration of the CPB procedure. A correlation was also found between the PBN adduct production and the concentration of creatine phosphokinase in muscle band of patients 1 h after the end of intervention. ROS circulating in the extracorporeal circuit and passing through heart during the reperfusion period could increase the myocardial damage related to ischemia-reperfusion. It has been shown both *in vitro* and *in vivo* that ROS,²⁻⁴ including alkyl-alkoxyl radicals trapped by PBN,^{9,26} could directly participate in cellular injury, resulting in depressed myocardial contractility. Therefore, the reperfused heart could not only be a site of production of ROS, but could also represent a target for systematically circulating activated cells producing ROS.²⁸⁻³⁰ This could be particularly relevant within the context of CPB, as high rates of ROS have been measured in the peripheral blood.

Human plasma protection against free radical injury is offered by a wide spectrum of antioxidants. We showed that the PAS was decreased during CPB, reflecting a reduced capacity of plasma to protect its environment from free radical aggressions, this phenomenon probably triggered in large part by hemodilution. On the other hand, in the current study, we did not show any consumption of antioxidant vitamins during the surgery despite of the demonstrated oxidative stress; however, another study conducted in our laboratory (unpublished data, 2000) showed that, 24 h after surgery, the vitamin C concentration was decreased. It seems that throughout surgery, the vitamin balance is stable, probably assisted by other antioxidants, whereas it could be affected after surgery. On the other hand, we observed an augmentation of the thiol concentration during CPB, which

has already been observed in some studies³¹ and which may be the result of a release or an increased transport of glutathione during specific conditions.³²

We observed an increase in uric acid concentration in peripheral blood samples during the CPB procedure, as previously described.³³ Systemic uric acid could derive from a cellular free radical attack on nucleic acid compounds or could be the result of restricted perfusion of peripheral tissues during CPB, leading to adenosine triphosphate and adenosine degradation. On the other hand, a significant difference was shown to arise between simultaneous CS and peripheral blood measurements at the beginning of reperfusion, reflecting a possible consumption of this compound by the myocardium, which has never been evidenced in humans. Uric acid has been reported to bear a potential protective physiologic function against the oxidative damage,³⁴ and it may be postulated that uric acid could have been consumed as an antioxidant by the myocardium within the firsts minutes of reoxygenation.

According to our results, it may be postulated that the peripheral oxidative stress we observed leads in large part to neutrophil activation.³⁵ It is known that the CPB procedure is associated with a major inflammatory response,^{36,37} potentially responsible for an activation of neutrophils that represents a prominent source of systemic primary oxygen free radical production.¹ The synergism of damages related to free radicals, activation and infiltration of neutrophils in reperfused tissues, has been well recognized for many years. Moreover, high oxygen tension used during CPB, concomitant with neutrophil activation, could also lead to oxidative stress.^{38,39} Some investigators recently suggested that future strategies of myocardial protection must not be limited to interventions targeted at the heart itself, but should take in account the systemic response of organism to CPB.^{30,38,40,41} These concepts should be particularly relevant for high-risk patients, who are more prone to organ injuries.

In conclusion, our investigations of oxidative stress during cardiac surgery through ESR spin-trapping studies demonstrated the occurrence of a free radical production during CPB. The systemic radical activation seems to be an important component of ROS release, which may overwhelm oxidative stress related to reperfusion of the ischemic myocardium, the circulating ROS being likely to participate in secondary myocardial damage. Our results suggest that strategies to improve patient outcome after CPB should encompass the systemic oxidative stress.

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