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Lipid peroxide and leukotriene B_4 production in patients undergoing cardiopulmonary bypass

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Abstract: Cardiac surgery with cardiopulmonary bypass (CPB) is frequently associated with a complex array of postoperative clinical abnormalities, including low-output syndrome and pulmonary dysfunction. It has been reported that oxygen free radicals are one of the important factors causing reperfusion injury. To determine whether oxygen free radicals are produced during cardiac surgery, we studied nine patients anesthetized with high doses of fentanyl. Lipid peroxide (LPO) and leukotriene B_4 (LTB_4) levels increased significantly from 60 min after aortic ligation to 180 min after reperfusion (aortic declamping), compared with the levels before surgery, while superoxide dismutase (SOD) was not affected markedly. Creatine kinase (CK), CK muscle-brain (CK-MB), and neutrophils increased from 60 min after aortic declamping. Correlations were not observed between LPO and CK nor between LPO and CK-MB. These results suggest that free radicals are generated during cardiac surgery with cardiopulmonary bypass (CPB), but it is unclear whether free radicals cause tissue injury after cardiac surgery with CPB.

Key words: Superoxide, Ischemia, Cardiac anesthesia

Introduction

Oxygen free radicals have recently been implicated as important agents of cellular damage after ischemia and reperfusion [1–7]. Neutrophils are known to accumulate at the site of infarction [8,9], and this fact suggests that they may be involved in the production of oxygen free radicals. There are many factors which produce oxygen free radicals in cardiac surgery with cardiopulmonary bypass (CPB). In the present study, we measured lipid peroxide (LPO) and superoxide dismutase (SOD) to investigate whether free radicals were formed during cardiac surgery with CPB. Electron spin resonance spectrometry detects free radicals directly, but it is not commonly used in clinical practice, where the formation of free radicals is proved indirectly by the increase in LPO. We also determined leukotrience B_4 (LTB_4) and platelet-activating factor (PAF) concentrations, both of which activate neutrophils strongly, and evaluated their participation in oxygen free radical production.

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Methods

With institutional approval and informed consent, we studied nine patients who underwent cardiac surgery (Table 1).

Preanesthetic medications included diazepam (0.2 $mg \cdot kg^{-1}$), hydroxyzine (1 $mg \cdot kg^{-1}$), pethizine (1 $mg \cdot kg^{-1}$), and atropine (0.01 $mg \cdot kg^{-1}$). Anesthesia was induced with fentanyl (30 $\mu g \cdot kg^{-1}$), and tracheal intubation was facilitated with vecuronium (0.15 $mg \cdot kg^{-1}$). Anesthesia was maintained using oxygen and high-dose fentanyl (total 100 $\mu g \cdot kg^{-1}$). Ventilation was controlled to maintain partial pressure of arterial carbon dioxide ($Paco_2$) at approximately 40 mmHg.

The perfusion apparatus included a hollow fiber membrane oxygenator (Capiox, Terumo, Tokyo, Japan) and nonpulsatile roller pump (Pemco, Cleviland, OH, USA). A mixture of 20% mannitol, 7% sodium bicarbonate, electrolyte solution, and citrate phosphate dextrose (CPD)-supplemented preserved blood was primed, and then perfused at a flow rate of 2.4 $l \cdot m^{-2} \cdot min^{-1}$. Hematocrit levels were maintained at 20% or more throughout CPB. Crystalloid cardioplegia was used for cardiac preservation. Modified GIK solution (15 $mg \cdot kg^{-1}$, every 30 min) was used for cardiac preservation and blood cardioplegia (10 $mg \cdot kg^{-1}$, every 30 min) with topical myocardial cooling.

The ECG, EEG, and esophageal and rectal temperatures were monitored continuously. Arterial blood

Table 1. Patient characteristics and results

Patient	Age	B.W. (kg)	Diag.	EF (%)	CPB (min)	Ao-clam. (min)	L-Temp. (c)	C-Plegia (ml)
1	60	46	AR	35	215	125	27.8	1900
2	64	53	AR + MR	35	215	94	27.0	1600
3	66	50	AP	56	220	80	27.0	1600
4	47	53	AR + MSR	52	234	231	26.0	2400
5	63	57	AP	56	155	80	27.0	1640
6	57	50	AR + MR	65	210	120	29.6	2300
7	67	55	AP	74	140	90	27.0	1750
8	50	57	AP	53	180	100	28.0	1500
9	72	65	AP	70	230	116	22.0	2450
Mean	60.7	53.9		55.1	199.9	115.1	26.8	1948.9
SD	8.12	5.52		13.70	33.60	46.53	2.07	360.74

B.W., body weight; Diag., diagnosis; EF, ejection fraction; CPB, cardiopulmonary bypass; Ao-clam., aortic clamping time; L-Temp., lowest temperature; C-plegia, cardioplegia; AR, aortic regurgitation; MR, mitral regurgitation; AP, angina pectoris; MSR, mitral stenosis and regurgitation.

oxygen saturation was also monitored continuously with a pulse oximter (Satlite, Datex, Helsinki, Finland) and end-tidal carbon dioxide concentrations with capnography (Capnomac, Datex). A catheter from which blood samples were drawn was placed in the radial artery to measure arterial pressure directly. Six blood samples were drawn after inducing anesthesia, at the following times: before surgery, immediately before starting CPB, 60min after aortic occlusion, and 60, 120, and 180min after aortic declamping. In each sample, serum creatine kinase (CK), CK muscle-brain (CK-MB), peripheral leukocyte, neutrocyte, LPO, SOD, LTB_4, and PAF concentrations were measured. LPO was determined by Ohkawa's method [10], SOD by the nitrite method [11], and LTB_4 and PAF by radioimmunoassay (RIA). Data were analyzed by analysis of variance (ANOVA) and Neuman-Keuls multiple comparison tests. Regression analysis was also used. A significant difference was defined as $P < 0.05$. All data are expressed as the mean ± SD.

Results

LPO increased significantly from 60min after aortic ligation, compared with preoperative levels after induction of anesthesia, and elevated levels were maintained up to 180min after reperfusion (aortic declamping) ($P < 0.01$, Fig. 1a). SOD tended to increase from 60min after aortic ligation, compared with preoperative levels, but the difference was not significant (Fig. 1b). The peripheral blood leukocyte and neutrophil counts increased significantly from 60min after reperfusion, compared with preoperative levels ($P < 0.01$, Table 2). LTB_4 increased significantly from 60min after aortic ligation, and peak levels appeared 60min after

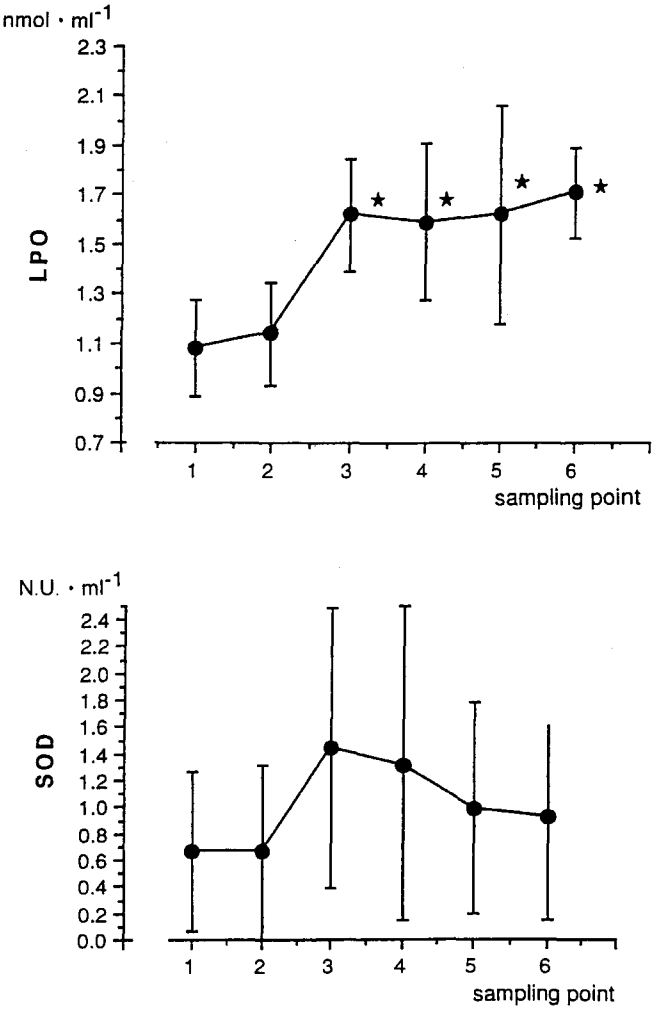

Fig. 1. a Changes in serum lipid peroxide (*LPO*) concentrations at the different time points (see "Method"). Values are shown as mean ± SD. *Asterisk*, $P < 0.01$ *vs* before surgery; $n = 8$. **b** Changes in serum superoxide dismutase (*SOD*) activity at the different time points (see "Method"). Values are shown as mean ± SD; the difference is not significant; $n = 8$

Table 2. Concentration of serum creatine phosphokinase (CK), CK-MB, and peripheral white blood cell (WBC) and polymorphonuclear leukocyte (PMN) counts at each time point

	1	2	3	4	5	6
CK ($IU \cdot l^{-1}$)	161 ± 116	229 ± 124	246 ± 73	428 ± 130*	519 ± 240*	592 ± 179*
CK-MB ($IU \cdot l^{-1}$)	0.5 ± 0.7	0.7 ± 0.9	0.7 ± 1.1	4.1 ± 3.2	6.9 ± 3.2*	10.9 ± 6.8*
WBC (μl^{-1})	5982 ± 2473	6392 ± 1963	5775 ± 1913	11275 ± 3801*	10569 ± 4729*	10715 ± 5075*
PMN (μl^{-1})	2717 ± 2421	3257 ± 2181	3016 ± 1786	7432 ± 4357*	7192 ± 4829*	7136 ± 5194*

Values are shown as mean ± SD. *$P < 0.01$ *vs* before surgery (1); $n = 9$.
Sampling points: (1) before surgery; (2) before CPB; (3) 60 min after aortic occlusion; (4) 60 min after reperfusion; (5) 120 min after reperfusion; (6) 180 min after reperfusion.

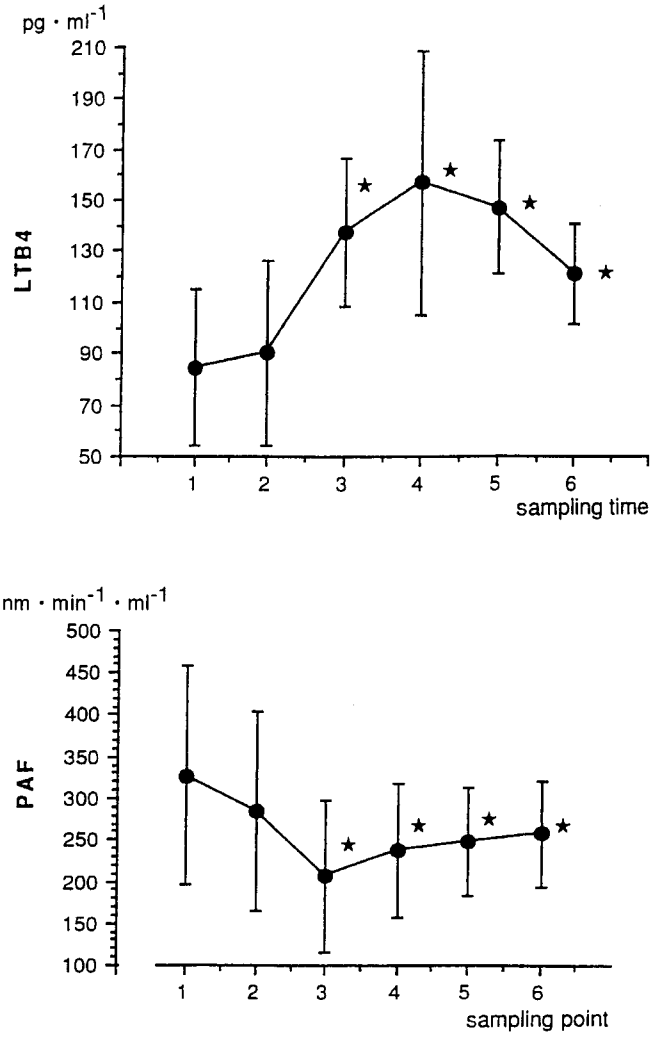

Fig. 2. a Changes in plasma leukotriene B_4 (LTB_4) levels at the different time points (see "Method"). Values are shown as mean ± SD. *Asterisk*, $P < 0.01$ *vs* before surgery; $n = 9$. **b** Changes in serum platelet activating factor (*PAF*) activity at the different time points (see "Method"). Values are shown as mean ± SD. *Asterisk*, $P < 0.01$ *vs* before surgery; $n = 9$

reperfusion. Elevated levels were maintained up to 180 min after reperfusion ($P < 0.01$, Fig. 2a). PAF decreased significantly from 60 min after aortic ligation, compared with preoperative levels ($P < 0.05$, Fig. 2b). CK and CK-MB increased significantly from 60 min after reperfusion. They increased in an almost linear fashion up to 180 min after reperfusion ($P < 0.01$, $P < 0.05$, Table 2). Correlations were not observed between LPO and CK-MB nor between LPO and CK.

Discussion

Oxygen free radicals such as superoxide anion (O_2^-), the hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2) have been implicated as agents of cellular damage in several disease processes, including ischemia and reperfusion of the heart [1–7]. In the present study, we found that LPO increased significantly from 60 min after aortic ligation up to 180 min after reperfusion (aortic declamping), compared with preoperative levels in patients who underwent cardiac surgery under cardiopulmonary bypass. SOD activity, however, tended to increase from 60 min after aortic ligation. These results led us to suspect that formation of free radicals had occurred. However, there were great variations with high standard deviations, and the changes were not statistically significant. We had expected that LPO would increase after reperfusion, but it actually began to increase before reperfusion. This might be explained by several factors, including (1) hyperoxia during CPB [12,13], (2) the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase reaction of activated neutrophils following complement activation in the pump oxygenator [14], (3) destruction of erythrocytes in the CPB circuit [15], and (4) the xanthine oxidase reaction [16]. During ischemia, oxidative phosphorylation by mitochondria is reduced, and ATP is degraded to hypoanthine, while xanthine dehydrogenase is converted to xanthine oxidase (XOD) and superoxide anion radical (O_2^-) is generated if oxygen is supplied by reperfusion. O_2^- is changed to H_2O_2 by SOD, and then further to H_2O by catalase or glutathione peroxidase (GSH Px). H_2O_2 reacts with Fe^{2+} in the body to form ·OH. These free radicals could be eliminated by various scavengers such as SOD, catalase, and GSH Px. However, if production exceeds elimination, unsaturated fatty acids in the

constitutive phospholipids of biological membranes are hyperoxidated, and this is followed by membrane degradation, which augments tissue injuries already induced by ischemia alone [17]. It has also been reported that neutrophils roll and stick to the vascular endothelium during ischemia and reperfusion, and they are activated, resulting in the formation of oxygen free radicals [18].

There is evidence that XOD activity is absent in the human heart, and many reports have shown that infarct size has been decreased in reperfusion models by suppressing neutrophil infiltration. As a result, attention has fucsed on the possible role of neutrophils in reperfusion injury [8,19,20]. Therefore, we measured the neutrophil count in the peripheral blood, as well as PAF and LTB_4, which are potent neutrophil activators. The neutrophil count increased significantly from 60 min after reperfusion. Similarly to LPO, LTB_4 began to increase from 60 min after aortic ligation and reached a high concentration at 60 min after reperfusion. This suggests that neutrophils might be activated by LTB_4. PAF fell significantly from 60 min after aortic ligation, compared with preoperative levels, suggesting that free radicals were produced by neutrophils activated by something other than PAF. CK-MB increased significantly from 60 min after reperfusion, compared with preoperative levels, but it is unclear whether free radicals participated in myocardial reperfusion injury in the present study because LPO did not correlate with CK-MB. In addition, LPO levels increased at 60 min after aortic occlusion, which shows that free radicals may be produced because of peripheral low-flow ischemia during CPB. Our previous study [21] had shown that interleukin 8 (IL-8), which has attracted attention as a potent neutrophil activator, increased during open heart surgery from 60 min after reperfusion, and that its changes correlated positively with granulocyte elastase and CK-MB. This suggested that lysosomes were released from neutrophils activated by IL-8, thus contributing to cytotoxicity. If activated neutrophils are one of the sources of free radical production, their possible relation to IL-8 is also an interesting topic for further research.

In conclusion, the present study showed an increase in LPO, suggesting an increased production of free radicals, during cardiopulmonary bypass.

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