Zhang SH *et al* / Acta Pharmacol Sin 2004 Mar; 25 (3): 334-340

Antioxidative effect of propofol during cardiopulmonary bypass in adults[1]

Shi-hai ZHANG[2], Shou-yong WANG, Shang-long YAO

Department of Anesthesiology, Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430022, China

KEY WORDS propofol; cardiopulmonary bypass; free radicals; F_2-isoprostanes; complement 5a

ABSTRACT

AIM: To investigate the antioxidative potential of propofol (an intravenous anesthetic with a chemical structure similar to phenol-based free radical scavengers such as vitamin E) during cardiopulmonary bypass (CPB). **METHODS:** Thirty adult patients referred for elective cardiac procedure with CPB were included and randomly allocated to a propofol group or a control group. Patients in the propofol group received propofol (0.1 mg·kg^{-1}·min^{-1}) intravenously for anesthesia maintenance, whereas those allocated to the control group received fentanyl 10 μg/kg intravenously and inhaled enflurane (1 %-1.5 %). Blood samples were collected at 7 time points: before the start of CPB, at 30 and 60 min of CPB, at the conclusion of CPB, 10 min after the administration of protamine, and 12 and 24 h after the cessation of CPB. Plasma levels of free F_2-isoprostanes (sensitive markers of free radicals production) and complement C5a were determined by mass-spectrometric assay and enzyme immunoassay, respectively. Neutrophil adhesion to endothelial cells was observed at ×200 magnification under a light microscope. **RESULTS:** Levels of F_2-isoprostanes, complement C5a and neutrophil adhesion rate increased significantly during and after CPB in both groups. There were significantly higher levels of F_2-isoprostanes, C5a, and more neutrophils adhering to endothelial cells in the control group than those in the propofol group, respectively. **CONCLUSION:** Cardiopulmonary bypass is associated with a great production of damaging free radicals. Propofol may be beneficial both as an anesthetic and as a potent free radical scavenger in patients presenting pathologies associated with free radical reactions during CPB.

INTRODUCTION

Cardiopulmonary bypass (CPB) can lead to the production of damaging oxygen-derived free radicals[1]. Some oxygen derivatives such as H_2O_2 and ·OH are considered to have potent oxidative activity to injure host tissue[2]. These reactive oxygen species cause lipid peroxidation of the cell membrane and intracellular Ca^{2+} overload, which are responsible for mechanical and metabolic damage [3]. Oxidant injury may be attenuated by endogenous antioxidant defenses. Antioxidants within cell membranes protect the membrane phospholipids from free radical-mediated lipid peroxidation. The best characterized of these is α-tocopherol (vitamin E)[4]. This compound contains a phenol group that donates hydrogen to free radicals, thus terminating lipid peroxidation.

[1] Project supported by National Natural Science Foundation of China (No 30271255).
[2] Correspondence to Shi-hai ZHANG. Phn 86-278-572-6834. Fax 86-278-572-6406. E-mail zhangshh@public.wh.hb.cn
Received 2003-04-23 Accepted 2003-10-21

Free radicals are difficult to quantify because of their high reactivity and very short life-spans. Prostaglandin F_2–like compounds (termed F_2-isoprostanes) may be good indicators of the extent of lipid degradation by free radicals. Morrow *et al*[5] have discovered a series of bioactive prostaglandin F_2-like compounds that are produced independently of the cyclooxygenase enzyme in humans by the peroxidation of arachidonic acid, catalyzed by free radicals. F_2-isoprostanes are initially formed in situ on phospholipids and are subsequently released. Levels of F_2-isoprostanes in normal human biologic fluids exceed those of cyclooxygenase-derived prostanoids by approximately one to two orders of magnitude. In addition, levels of F_2-isoprostanes, both free in the circulation and esterified to tissue phospholipids, increase dramatically in animal models of oxidant injury[6]. F_2-isoprostanes are therefore recognized as markers of free radicals production.

During CPB, the activated complement system (such as C5a) interacts with receptors on the neutrophils and induces leukocyte chemotaxis, autoaggregation, increased adherence, and superoxide generation[7]. Neutrophil activation results in the formation of a large quantities of oxygen free radicals (superoxide anion and hydroxyl radicals) and reactive oxygen species, which can be released into the external tissue environment[8]. Therefore, neutrophil activation and plasma C5a play important roles in free radicals production and oxidative damage.

Propofol, with a phenol structure, has been used extensively as a rapidly acting intravenous anesthetic for inducing and maintaining general anesthesia. The antioxidant potential of propofol has never been well evaluated. We undertook this study to assess the hypothesis that propofol can inhibit oxidative modification of important biologic molecules in humans by determining whether the production of F_2-isoprostanes is decreased and the outcome improved in CPB persons who are administered propofol as an intravenous anesthetic for anesthesia maintenance.

MATERIALS AND METHODS

Patients and anesthesia Thirty patients scheduled for elective cardiac surgery with CPB were included in this study. Patients with hepatic and renal dysfunction, and those having received steroids drugs in the previous 2 weeks were excluded. Smokers were also excluded, for smokers have increased circulating F_2-isoprostanes levels[9]. The patients were divided randomly into a propofol group and a control group, with 15 patients in each group. Patient and intraoperative data are summarized in Tab 1.

Anesthesia and CPB technique Diazepam and scopolamine were used for premedication and anesthesia was induced with fentanyl (20 μg/kg). Muscle relaxation was achieved with vecuronium (0.1 mg/kg). For anesthesia maintenance of the patients in the control group, the lungs were ventilated with low concentrations of enflurane (1.2 %-1.8 %) in pure oxygen until shortly before CPB was started, when enflurane inhalation was discontinued. Fentanyl 10 μg/kg was used during CPB. Propofol (0.1 $mg \cdot kg^{-1} \cdot min^{-1}$) was administered for anesthesia maintenance of the patients in the propofol group after tracheal intubation.

Heparin 4 mg/kg was used as anticoagulant agent. The activated clotting time (ACT) was maintained >480 s during CPB. CPB was performed with nonpulsatile flow of 2.4 L/min per BSA (body surface area) with a Sarns 9000 roller-pump (Sarns/3M, Ann Arbor, Mich) and membrane oxygenator. Patients were cooled down to 34 ºC (nasopharyngeal) at the beginning of CPB and rewarmed to 36 ºC before weaning from bypass. After CPB, heparin was neutralized with protamine sulfate until the preoperative ACT was achieved.

Blood samples Blood samples were collected at 7 points of time: (1) before the start of CPB, (2,3) at 30 and 60 min of CPB, (4) at the conclusion of CPB, (5) 10 min after the administration of protamine, and (6,7) 12 and 24 h after the cessation of CPB. Samples were obtained from the arterial line before (heparinized blood) and after CPB (heparin used as anticoagulant). During CPB, samples were obtained from the arterial limb of the CPB circuit.

Measurements of F_2-isoprostanes Plasma F_2-isoprostanes were extracted with one-step extraction procedure[10] and plasma free F_2-isoprostanes measured with gas chromatography-mass spectrometry[11]. Briefly, heparinized blood sample (4 mL) was collected into a tube containing indomethacin, an inhibitor of cyclooxygenase, at a final concentration of 15 μmol/L. The blood tube was centrifuged at 2400×*g* at 4 ºC for 10 min to separate plasma and erythrocytes. Aliquots of plasma (1 mL) were transferred to the Eppendorf tubes containing butylated hydroxytoluene, a scavenger of free radicals, at a final concentration of 20 μmol/L. One milliliter of 1 mol/L KOH was added to the Eppendorf tube containing the plasma sample. The plasma sample

was hydrolyzed at 40 ºC for 30 min to release bound (esterified) lipids. At the end of the hydrolysis, 1 mL of 1 mol/L HCl and 2 mL of 100 mmol/L formate buffer (pH 3.0) were added. The sample was centrifuged at 2400×*g* for 10 min; the supernatant was then ready for solid-phase extraction. The sample was then applied to an Oasis HLB extraction cartridge preconditioned with methanol and 10 mmol/L formate buffer (pH 3.0). The cartridge was washed with 5 mL of the formate buffer followed by 5 mL of acetonitrile-water (15:85 by volume). F_2-isoprostanes were eluted by washing the cartridge with 2 mL of hexane-ethyl acetate-propan-2-ol (30:65:5 by volume). F_2-isoprostanes obtained by the purification procedures were analyzed by gas chromatography-mass spectrometry[11]. Data are expressed in picomoles per liter (pmol/L).

Complement C5a assay All blood samples were immediately evaluated and spun at 2000×*g* for 15 min; the plasma was separated and frozen at -70 ºC. Complement C5a was measured by enzyme immunoassay.

Neutrophil adhesion to endothelial cells Human pulmonary artery endothelial cells (HPAECs) were cultured with a standard method[12]. One hundred microliters of isolated neutrophils (5×10^5 cells/mL) from the propofol or control group were applied to HPAEC monolayers in six-well tissue culture plates in a humidified multigas incubator for 48 h. Nonadherent neutrophils were removed by gently washing the plates three times with prewarmed Dulbecco's phosphate-buffered saline. Ten randomly selected fields were read at ×200 magnification under an optical microscope. Neutrophil adhesion rate was evaluated by counting the number of neutrophils adhering to the endothelial cell monolayer. Data are expressed in cells per mm^2 (cells/mm^2).

Statistical analysis All values are expressed as mean±SD. Comparisons between groups were made by means of two-way analysis of variance for repeated measurements. Comparisons within groups were performed by means of one-way analysis of variance followed by the Tukey test for multiple comparisons.

RESULTS

Clinical parameters The groups did not differ in sex, age, severity of disease, CPB time, and aortic cross-clamping time (Tab 1).

F_2-isoprostanes levels Before CPB, free F_2-isoprostanes levels were similar in both groups. Significant increases of free F_2-isoprostanes were observed in both groups during and after CPB. Compared with those in the propofol group, the levels of free F_2-isoprostanes in the control group were significantly higher at 30 and 60 min of CPB, at 12 h and 24 h after CPB cessation, and most significantly at the end of CPB and at 10 min after protamine administration, respectively. The changes of free F_2-isoprostanes levels were shown in Fig 1.

C5a concentrations Plasma concentrations of C5a increased sharply in both groups compared to the respective value before CPB. Compared with those in the propofol group, plasma concentrations of C5a in the control group increased most significantly at 30 and 60 min of CPB, at the end of CPB, at 10 min after protamine administration, at 12 h and 24 h after CPB cession, respectively (Fig 2).

Neutrophil adhesion rate Before CPB, there was no difference of neutrophil adhesion rate between two groups (Fig 3). There was a dramatic increase of neutrophil adhesion rate in both groups during and after CPB. In contrast, a much higher increase of neutrophil adhesion rate was observed in the control group than that in the propofol group during and after CPB.

Tab 1. Patient characteristics and intraoperative data.

	Clinical parameters						
	Sex		Age/year	MVR	MVR +AVR	CPB/min	ACT/min
	M	F					
Control	11	4	50.2±8.9	8	7	142.5±49.4	61.6±21.9
Propofol/0.1 mg·kg^{-1}·min^{-1}	10	5	48.2±10.8	11	4	120.3±38.9	50.6±42.8

M, Male; F, female; MVR, mitral valve replacement; AVR, aortic valve replacement; CPB, cardiopulmonary bypass; ACT, aortic-clamping time.

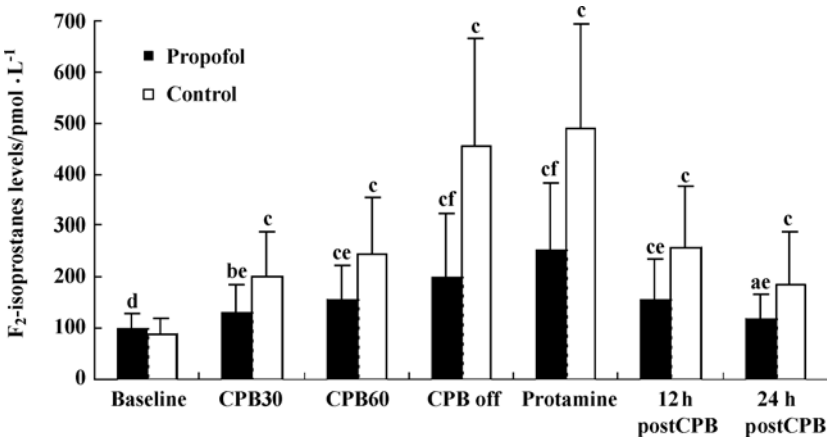

Fig 1. Plasma levels of F_2-isoprostanes before, during, and after CPB in the propofol group and in the control group. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* baseline; [d]*P*>0.05, [e]*P*<0.05, [f]*P*<0.01 *vs* control group.

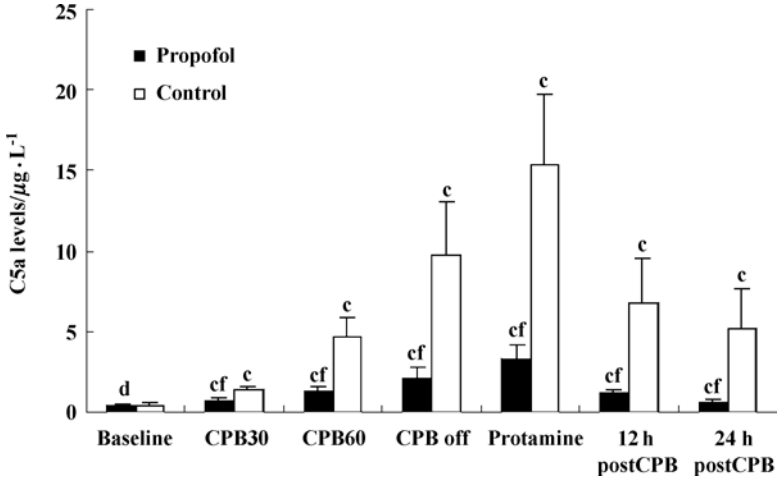

Fig 2. Plasma levels of complement C5a before, during, and after CPB in the propofol group and in the control group. Mean±SD. [c]*P*<0.01 *vs* baseline; [d]*P*>0.05, [f]*P*<0.01 *vs* control group.

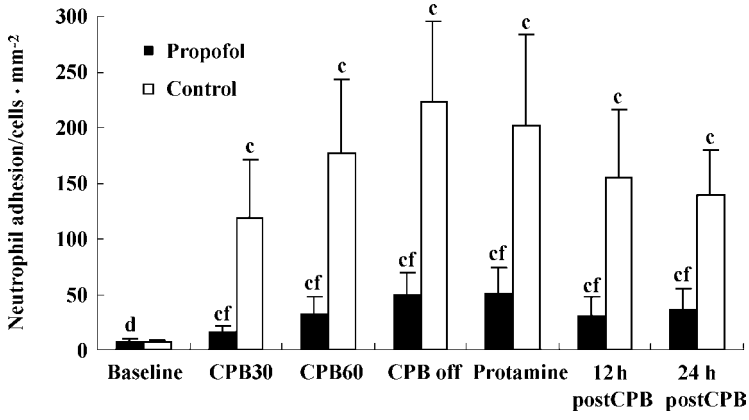

Fig 3. Neutrophil adhesion to endothelial cells before, during, and after CPB in the propofol group and in the control group. Mean±SD. [c]*P*<0.01 *vs* baseline; [d]*P*>0.05, [f]*P*<0.01 *vs* control group.

DISCUSSION

Although free radicals contribute to many damaging states, their role is difficult to quantify. Because free radicals themselves are highly reactive, with life spans of the order of microseconds for most oxygen-derived radicals, they can be measured only directly by electron spin resonance spectroscopy[13]. At present this can be applied only to small samples of tissues and to cell free system. Thus direct measurement of free radicals has been possible only *in vitro* and in some animal experiments[14]. Isoprostanes belong to a family of free radical-catalyzed products of arachidonic acid. F_2-isoprostanes are formed in response to oxidative stress, initially *in situ*, via the formation of peroxyl radicals of arachidonic acid in the phospholipid. These compounds are good indicators of the extent of lipid degradation because they are specific, chemically stable and may have significant biological activities[15].

Our finding that the production of F_2-isoprostanes is higher in the patients of the control group than those in the propofol group provides compelling evidence that propofol attenuates oxidative modification of biologic components in humans during CPB. This conclusion is greatly strengthened by the findings that levels of C5a and neutrophil adhesion rate in the patients of the propofol anesthesia group was significantly lower than those in the control group. These results provide a basis for hypotheses that link the antioxidative effects of propofol to its specific chemical structure. Free radicals, especially the hydroxyl radical generated from H_2O_2, can easily remove a hydrogen atom from a methylene carbon of an unsaturated fatty acid of membrane phospholipids[16]. Peroxidation of lipids may inactivate membrane-associated enzymatic proteins, such as Na^+-K^+-adenosinetriphosphatase and Ca^{2+}-adenosinetriphosphatase[17]. These alterations of the cell membrane may be responsible for intracellular Ca^{2+} over-load. Intracellular Ca^{2+} accumulation may cause myocardial contracture[18]. Propofol inhibits the slow inward L-type Ca^{2+} current across the sarcolemma[19]. Propofol contains a phenol group that donates hydrogen to free radicals, thus terminating lipid peroxidation.

However, the results of previous studies of propofol and its antioxidative effect in humans have been conflicting and difficult to interpret. Whether therapeutic concentration of propofol ameliorates free radicals or not is controversial. Green *et al*[19] reported that the concentration of propofol required to ameliorate free radicals is approximately an order of magnitude higher than therapeutic doses of propofol used in anesthesia and suggests that its scavenging activity during anesthesia is likely very limited. Murphy *et al*[20] revealed that the free radical scavenging properties of propofol are biologically significant at anesthetic doses. The reason for these discrepancies may be that the measurement of oxidative products is not an accurate indicator of free radicals in biologic samples. Morrow *et al*[9] have shown that circulating levels of F_2-isoprostanes appear to provide an accurate measure of lipid peroxidation *in vivo*. Thus, the finding in this study that plasma F_2-isoprostanes levels from patients in the propofol group were decreased by propofol administration may provide a clinical link between clinical concentration of propofol and free radical scavenging.

It was surprising that blood F_2-isoprostanes levels were higher 24 h postoperatively than that before CPB in the control group. One would have been expected that F_2-isoprostanes would be normal 24 h postoperatively because of quick elimination of F_2-isoprostanes from urine. The phenomenon is hard to explain at the moment. We can speculate that the release of free radicals from neutrophils might be responsible for the high F_2 isoprostanes 24 h postoperatively[21]. Polymorphonuclear leukocyte activation results in the formation of a large quantities of oxygen free radicals during CPB, the oxygen free radical-producing activity of polymorphonuclear leukocyte is still high 24 h postoperatively[21].

In this study, lower levels of complement C5a in the propofol group compared with those in the control group indicates less free radicals release from neutrophils in the presence of propofol. The use of CPB is associated with a systemic inflammatory response. Because of the exposure to a large foreign body, systemic endotoxemia occurs and is followed by a release of inflammatory and anti-inflammatory cytokines[22]. Complement activation of the alternative pathway is reflected by an increase of C5a, which is part of the acute-phase immune response. When CPB is used, this acute-phase response is significantly augmented. Furthermore, the early increase of C5a after the onset of CPB demonstrates a potential triggering mechanism through contact with the artificial surface of the oxygenator tubes and filters. As pointed out by Vertrees *et al*[23], activation of the complement cascade and the increase of inflammatory immune regulators, such as IL-6, IL-8, and TNF-α, cause a release of neutrophil leukocytes from the bone marrow as part of the acute-phase

reaction. During CPB, the activated complement system (C5a) interacts with receptors on the neutrophils and induces leukocyte chemotaxis, autoaggregation, increased adherence, and superoxide generation[7]. Neutrophil activation results in the formation of a large quantities of oxygen free radicals (superoxide anion and hydroxy radicals) and reactive oxygen species, which can be released into the external tissue environment[7,8]. Therefore, inhibition of neutrophil activation may reduce the production of free radicals during CPB. Mikawa and colleagues[24] have reported that propofol inhibits chemotaxis, phagocytosis, and reactive oxygen species production from neutrophils. Suppression of the increase in $[Ca^{2+}]_i$ of neutrophils in response to stimulus may be one mechanism responsible for the inhibition of neutrophil functions by propofol[24]. The inhibition of neutrophil function by propofol may explain, at least in part, the reduction of free radicals production in the presence of propofol in this study. The mechanism of complement activation inhibition by propofol remains poorly unknown at the moment.

Our findings that C5a levels and neutrophil adhesion rate increased markedly in the patients of the control compared with those in the propofol group indicates the improved outcome of the patients by propofol administration. CPB treatment of surgical patients is a model of cell-mediated oxidative stress because it leads to the activation of platelets and neutrophils[25]. Neutrophil activation is primarily caused by endothelial interaction during ischemia. On reperfusion, activated neutrophils are flushed from pulmonary and cardiac capillaries into the systemic circulation. Accordingly, the reperfusion period is shown to entail an increase in circulating concentrations of free radicals[26]. These investigations have shown the peak concentration of free radicals in the peripheral circulation system appears at the reperfusion period. Our findings by measurements of plasma levels of F_2 isoprostanes, C5a and neutrophil adhesion are in agreement with their reports. The phenomenon implicates that using antioxidants before reperfusion may be the best time.

Free radical activity may play an important role in the pathogenesis of myocardium and lung injury. Reduction of free radicals may improve outcomes of patients undergoing cardiac surgery with CPB. Common antioxidants such as vitamin E and butylated hydroxytoluene cannot be used routinely due to their serious side effects. During CPB, anesthesia is needed to suppress vicious reflexes and stress. Propofol is the first candidate because of its rapid acting and recovering. Propofol has a chemical structure similar to vitamin E and butylated hydroxytoluene. Our findings demonstrate that propofol can be beneficial both as an anesthetic and as a free radical scavenger in patients presenting pathologies associated with free radical reactions during CPB.

ACKNOWLEDGEMENT The authors express their gratitude to Dr Qing-hua HU, Department of Cardiology, The Johns Hopkins University, for his critical review of the manuscript.

REFERENCES

1 Cavarocchi NC, England MD, Schaff HV, Russo P, Orszulak TA, Schnell WA Jr, *et al*. Oxygen free radical generation during cardiopulmonary bypass: correlation with complement activation. Circulation 1986; 74 (suppl): III 130-3.

2 Nakaya H, Tohse N, Kanno M. Electrophysiological derangements induced by lipid peroxidation in cardiac tissue. Am J Physiol 1987; 253: H1089-97.

3 Webster NR, Nunn JF. Molecular structure of free radicals and their importance in biological reactions. Br J Anaesth 1988; 60: 98-108.

4 Navapurkar VU, Skepper JN, Jones JG, Menon DK. Propofol preserves the viability of isolated rat hepatocyte suspensions under an oxidant stress. Anesth Analg 1998; 87: 1152-7.

5 Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ II. A series of prostaglandin F_2-like compounds are produced *in vivo* in humans by a noncyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 1990; 87: 9383-7.

6 Morrow JD, Awad JA, Kato T, Takahashi K, Badr KF, Roberts LJ II, *et al*. Formation of novel non-cyclooxygenase-derived prostanoids (F_2-isoprostanes) in carbon tetrachloride hepatotoxicity: an animal model of lipid peroxidation. J Clin Invest 1992; 90: 2502-7.

7 Prasad K, Kalra J, Bharadwaj B, Chaudhary A. Increased oxygen free radical activity in patients on cardiopulmonary bypass undergoing aortocoronary bypass surgery. Am Heart J 1992; 123: 37-45.

8 Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reaction. Am J Pathol 1982; 47: 397-417.

9 Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, *et al*. Increase in circulating products of lipid peroxidation (F2-Isoprostanes) in smokers - smoking as a cause of oxidative damage. N Engl J Med 1995; 332: 1198-203.

10 Zhao Z, Lam CWK, Ho CS. One-step solid-phase extraction procedure for F_2-isoprostanes. Clin Chem 2001; 47:1306-8.

11 Morrow JD, Roberts LJ II. Mass spectrometry of prostanoids: F_2-isoprostanes produced by non-cyclooxygenase

free radical-catalyzed mechanism. Methods Enzymol 1994; 233:163-74.

12 Suzuki Y, Nishio K, Takeshita K, Takeuchi O, Watanabe K, Sato N, *et al*. Effect of steroid on hyperoxia-induced ICAM-1 expression in pulmonary endothelia cells. Am J Physiol Lung Cell Mol Physiol 2000; 278: L245-52.

13 Murphy PG, Davies MJ, Columb MO, Stratford N. Effect of propofol and thiopentone on free radical mediated oxidative stress of the erythrocyte. Br J Anaesth 1996; 76: 536-43.

14 Davies SW, Duffy JP, Wickens DG, Underwood SM, Hill A, Alladine MF, *et al*. Cardiopulmonary bypass, myocardial management, and support techniques: time-course of free radical activity during coronary artery operations with cardiopulmonary bypass. J Thorac Cardiovasc Surg 1993; 105: 979-87.

15 McIntyre TM, Zimmerman GA, Prescott SM. Biologically active oxidized phospholipids. J Biol Chem 1999; 274: 25189-92.

16 Hess ML, Manson NH. Molecular oxygen: Friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J Mol Cell Cardiol 1984; 16: 969-85.

17 Corretti MC, Koretsune Y, Kusuoka H, Chacko VP, Zweier JL, Marban E. Glycolytic inhibition and calcium overload as consequences of endogenously generated free radicals in rabbit hearts. J Clin Invest 1991; 88: 1014-25.

18 Cook DJ, Housmans PR. Mechanism of the negative inotropic effect of propofol in isolated ferret ventricular myocardium. Anesthesiology 1994; 80: 859-71.

19 Green TR, Bennett SR, Nelson VM. Specificity and properties of propofol as an antioxidant free radical scavenger. Toxicol Appl Pharmacol 1994; 129: 163-9.

20 Murphy PG, Bennett JR, Meyers DS, Davies MJ, Jones JG. The effect of propofol anaesthesia on free radical-induced lipid peroxidation in rat liver microsomes. Eur J Anaesthesiol 1993; 10: 261-6.

21 Chenoweth DE, Hugli TE. Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. Proc Natl Acad Sci USA 1978; 75: 3943-7.

22 Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD. Complement and the damaging effects of cardiopulmonary bypass. J Thorac Cardiovasc Surg 1983; 86:845-57.

23 Vertrees R, Tao W, Kramer GC, Nutt L, McDaniel LB, DeVine SD, *et al*. Tumor necrosis factor monoclonal antibody prevents alteration in leucocyte population during cardiopulmonary bypass. ASAIO J 1994;40: M554-9.

24 Mikawa K, Akamatsu H, Nishina K, Shiga M, Maekawa N, Obara H, *et al*. Propofol inhibits human neutrophil functions. Anesth Analg 1998; 87: 695-700.

25 Hall RI, Smith MS, Rocker G. The systemic inflammatory response to cardiopulmonary bypass: pathophysiological, therapeutic, and pharmacological considerations. Anesth Analg 1997; 85: 766-82.

26 Rashid MA, William-Olsson G. Are leukocytosis and lipid peroxidation involved in ischemic or reperfusion injury in cardiac surgery? Thorac Cardiovasc Surg 1991; 39: 193-5.