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Deleterious Effects of Hyperoxemic Extracorporeal Circulation during Cardiovascular Surgery

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Although extracorporeal circulation (ECC) has been routinely used for cardiovascular surgery, hyperoxemia during ECC may produce oxygen toxicity and cellular injury. This study was performed to investigate the clinical influences of hyperoxemic ECC during cardiovascular operation. 40 adult patients scheduled for elective cardiovascular surgery were classified into normoxemic (arterial oxygen tension: 115 mmHg, n=20) and hyperoxemic (arterial oxygen tension: 380 mmHg, n=20) ECC. At preoperative and postoperative period, total leukocyte and neutrophil counts, platelet counts, iron, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine in peripheral arterial blood, malondialdehyde (MDA) and troponin-T concentration (TnT) in coronary sinus blood, pulmonary vascular resistance (PVR), and postoperative blood loss volume (BLS) were measured and compared between groups. Hyperoxemic group had postoperatively higher total leukocyte and neutrophil counts, MDA, TnT, PVR, total BLS, iron, glucose, AST, ALT, BUN, and creatinine than normoxemic group (p<0.05). In conclusion, hyperoxemic ECC results in greater inflammatory response and oxidative damaging effects on the heart, lung, liver and kidney, probably being adverse to postoperative patient recovery. For reducing these deleterious effects and improving postoperative outcomes, management lowering oxygen tension during ECC is recommended.

Key Words: Cardiovascular surgery, Extracorporeal circulation, Oxygen tension

INTRODUCTION

Extracorporeal circulation (ECC) for cardiovascular operations had been recognized as a safe clinical technique and usually used. Hemodilution technique (hematocrits of 20~25%) is necessarily used for improving peripheral circulation during ECC. The hemodilution may contribute to less oxygen delivery to the whole body, and thus arterial oxygen tension (PaO_2) during ECC is managed with a hyperoxemic condition (about 300 to 500 mmHg), probably producing much oxygen-derived free radical (cytotoxic oxygen metabolite). Cytotoxic oxygen metabolites, such as superoxide anion ($O_2^-\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\cdot$), play an important role in the genesis of postischemic dysfunction[4]. Furthermore, hyperbaric oxygen accelerates the effects of oxygen toxicity and also damages the central nervous system, probably because of the higher partial pressures of arterial oxygen.[13] Several recent studies have reported that hyperoxemic ECC may lead to a myocardial reoxygenation injury in hypoxic immature heart and adverse effects in the adult cardiac surgery[2,34,36,45]. The investigators have asserted the beneficial effects of normoxemic ECC. However, most of these findings are limited on the heart and lung and there is a few information about the effect of hyperoxemic ECC on the whole body. Much information on the influence of hyperoxemic ECC should be accumulated because ECC is an essential procedure for cardiovascular surgery. This study was carried out to investigate the clinical influences of hyperoxemic ECC on the whole body, including the heart, lung, liver, kidney and hematology.

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MATERIALS AND METHODS

1. Patients

40 adult patients scheduled for elective cardiovascular surgery were participated in this study. They were randomly classified into normoxemic (PaO_2: about 115 mmHg,

n=20) and hyperoxemic (PaO_2: about 380 mmHg, n=20) group with the surgeon and the coronary care unit (CCU) staff blinded to the assignment. The patients with preoperative disorders (e.g., diabetes mellitus, liver, kidney, or lung disorders) were excluded in this study.

2. Extracorporeal circulation and myocardial protection

The extracorporeal circulation was performed using a Stockert 5-roller pump (Stockert, Munch, Germany) and nonpulsatile flow at rates of 1.8~2.5 L/min/m^2 with UNIVOX membrane oxygenator (Baxter Healthcare Co., USA) and GISH tubing pack (GISH Co., USA). Moderate hypothermia at 31~32℃ was instituted immediately after the start of ECC. Myocardial protection was achieved with antegrade/retrograde blood cardioplegic arrest. Normoxemic group underwent normoxic (PaO_2: range 95~140 mmHg) ECC and hyperoxemic group underwent hyperoxic (PaO_2: range 340~450 mmHg) ECC. Hematocrit was maintained ≥20% on ECC, with the addition of blood as necessary. All patients were weaned from ECC at rectal temperature of 35.5℃ and protamine was administered for heparin reversal. The demographic characteristics except partial pressures of arterial oxygen (PaO_2) were not significantly different between the two groups (Table 1).

Table 1. Demographic charateristics of the two groups

Characteristics \ Group	Normoxemic group	Hyperoxemic group
No. of patients	20	20
Age (year)	49.7±5.8	51.2±5.3
Weight (kg)	65.7±5.6	64.8±6.2
BSA (m^2)	1.70±0.06	1.67±0.06
Perfusion rate (L/min/m^2)	2.13±0.05	2.11±0.06
Hypothermia (℃)	31.5±0.6	31.6±0.5
ACT (min)	89.6±6.5	90.2±5.3
TBT (min)	126.3±8.6	130.4±11.5

Data were expressed as mean ± standard error (SE).
There was no difference in the characteristics between the two groups (p>0.05).
Legend: BSA, body surface area; ACT, aortic-cross clamping time; TBT, total bypass time

3. Sampling and measurements

Samples of peripheral blood were taken from the radial artery. For hematological evaluation, total leukocyte, neutrophil and platelet counts were measured at preoperative and postoperative period. For assessment of biochemical markers (metabolism, liver function, and kidney function), iron, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine level were determined before and after operation. Retrograde cardioplegic catheter was inserted into coronary sinus of the heart and coronary sinus blood was drawn for measurement of malondialdehyde (MDA) and troponin-T concentration (TnT) as myocardial oxidative and injury marker at the beginning and end of operation. Swan-Ganz catheter was inserted into pulmonary artery via internal jugular vein and pulmonary vascular resistance (PVR) as a lung injury marker was measured before and after operation. Blood loss volume was counted at postoperative 24 h, 48 h, 72 h and total periods in CCU.

The amounts of iron, glucose, AST, ALT, BUN and creatinine were determinated using test kits with RXL instrument (Dade Behring Co., USA). MDA was determined by test kit (BIOXTECH LPO-586 Assay, Oxis International Inc. USA) with Diode Array Spectrophotometer (HP8452A, Hewlett packard, USA) and TnT was measured by test kit with Elecsys 2010 (Boehringer Mannheim Co., Germany). All parameters were compared between groups.

4. Statistical analysis

Differences between preoperative and postoperative parameters (total leukocyte and neutrophile counts, platelet counts, iron, glucose, AST, ALT, BUN, creatinine, MDA, TnT and PVR) in each group were evaluated by paired t-test. Unpaired t-test was used for comparing differences between groups. Data were analyzed in a statistics program (SAS version 6.03) on a computer and statistical significance was accepted with $p \leq 0.05$. All data were expressed as mean±standard error (SE).

RESULTS

1. Total leukocyte

Total leukocyte counts in both groups at postoperative period (normoxemic=10,420±790/mm^3 vs hyperoxemic =13,700±1,065/mm^3) were elevated compared with preoperative period (normoxemic=6,040±420/mm^3 vs hyperoxemic=6,300±530/mm^3). Postoperative value was higher

in hyperoxemic group ($p < 0.05$) (Fig. 1).

2. Neutrophil

Neutrophil counts in both groups at postoperative period (normoxemic=8,370±890/mm^3 vs hyperoxemic=10,620± 1,180/mm^3) increased compared with preoperative period (normoxemic=4,035±310/mm^3 vs hyperoxemic=4,185± 378/mm^3). Postoperative value in hyperoxemic group was significantly higher than that in normoxemic group ($p<$ 0.05) (Fig. 2).

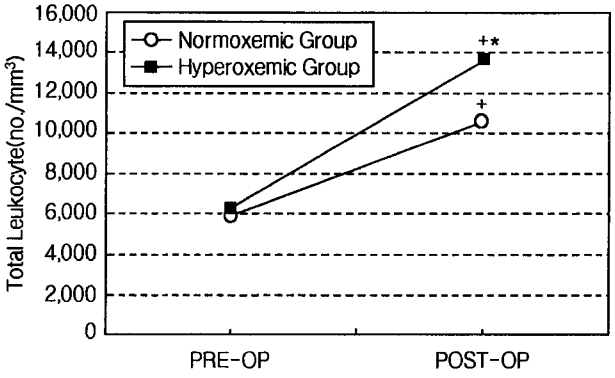

Fig. 1. Total leukocyte counts in peripheral blood in normoxemic and hyperoxemic group at preoperative and postoperative period. Total leukocyte counts at postoperative period were higher in hyperoxemic group compared with normoxemic group, suggesting that hyperoxemia generates a greater inflammatory reaction than normoxemia (+, $p<0.05$ compared with PRE-OP; *, $p<0.05$ compared with normoxemic group).

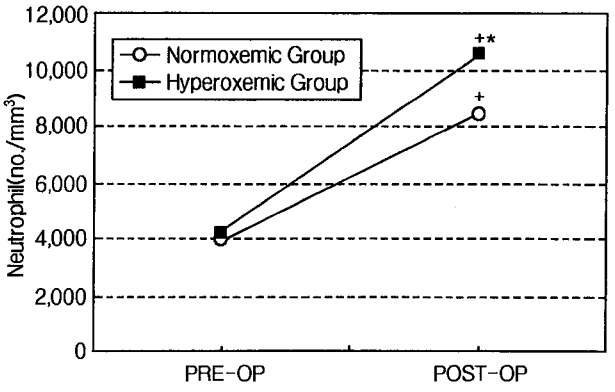

Fig. 2. Neutrophil counts in peripheral blood in normoxemic and hyperoxemic group at preoperative and postoperative period. Neutrophil counts at postoperative period were higher in hyperoxemic group compared with normoxemic group. These elevations contribute to increase of total leukocyte counts (+, $p<0.05$ compared with PRE-OP; *, $p<0.05$ compared with normoxemic group).

3. Platelet

Platelet counts in both groups decreased at postoperative period (normoxemic=145,860±6,578/mm^3 vs hyperoxemic =156,700±10,736/mm^3) compared with preoperative period (normoxemic=213,500±13,389/mm^3 vs hyperoxemic =241,260±16,404/mm^3). However, postoperative value did not differ between groups ($p > 0.05$) (Fig. 3).

4. Malondialdehyde

In both groups, malondialdehyde concentrations (MDA) significantly increased at postoperative period (normoxemic

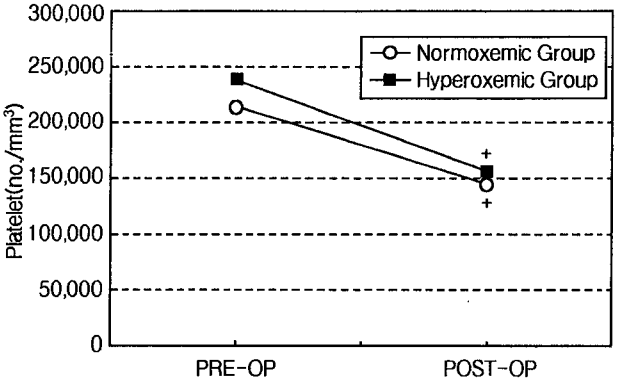

Fig. 3. Platelet counts in peripheral blood in normoxemic and hyperoxemic group at preoperative and postoperative period. There was no significance between groups (+, $p<0.05$ compared with PRE-OP).

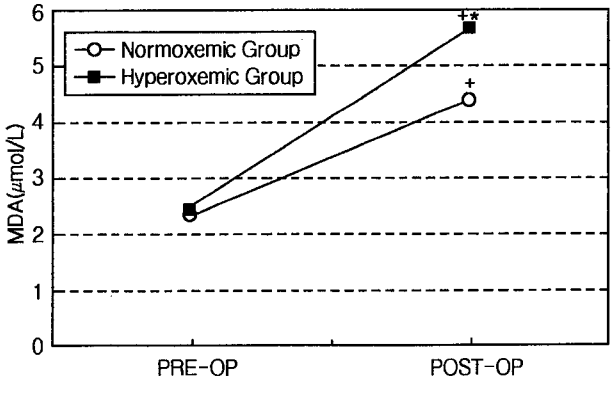

Fig. 4. Malondialdehyde concentrations (MDA) in coronary sinus blood in normoxemic and hyperoxemic group at preoperative and postoperative period. MDA at postoperative period was high in hyperoxemic group compared with normoxemic group, indicating that hyperoxemia causes more severe oxidative stress for the myocardium (+, $p<0.05$ compared with PRE-OP; *, $p<0.05$ compared with normoxemic group).

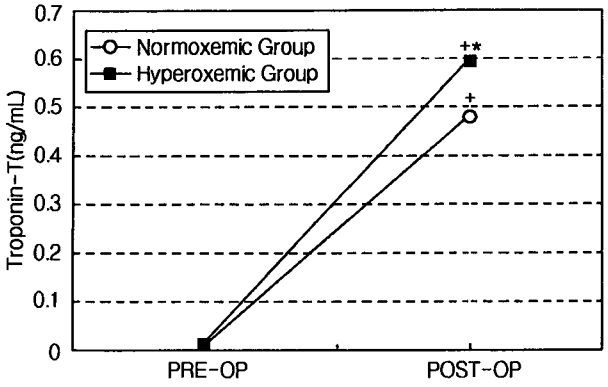

Fig. 5. Troponin-T concentrations (TnT) in coronary sinus blood in normoxemic and hyperoxemic group at preoperative and postoperative period. Hyperoxemic group had higher TnT than normoxemic group at postoperative period, implying that hyperoxemia produces more severe myocardial injury than normoxemia (+, $p<0.05$ compared with PRE-OP; *, $p<0.05$ compared with normoxemic group).

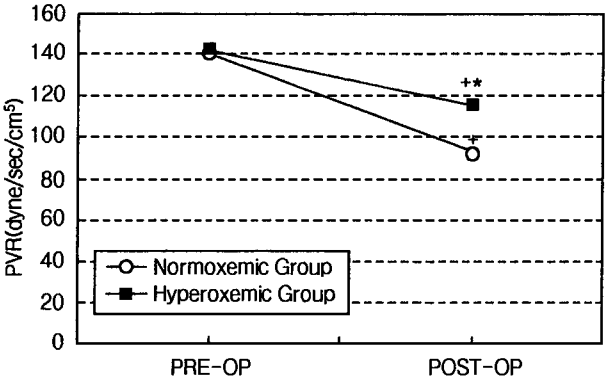

Fig. 6. Pulmonary vascular resistance (PVR) in normoxemic and hyperoxemic group at preoperative and postoperative period. PVR at postoperative period was high in hyperoxemic group compared with normoxemic group, meaning that hyperoxemia generates more severe pulmonary injury than normoxemia (+, $p<0.05$ compared with PRE-OP; *, $p<0.05$ compared with normoxemic group).

=4.38±0.70 μmol/L vs hyperoxemic=5.65±0.63 μmol/L) compared with preoperative period (normoxemic=2.37± 0.16 μmol/L vs hyperoxemic=2.43±0.19 μmol/L). Postoperative value in hyperoxemic group was significantly higher than that in normoxemic group ($p<0.05$) (Fig. 4).

5. Troponin-T

In both groups, troponin-T concentrations (TnT) were elevated at postoperative period (normoxemic=0.48±0.09 ng/mL vs hyperoxemic=0.59±0.09 ng/mL) compared with

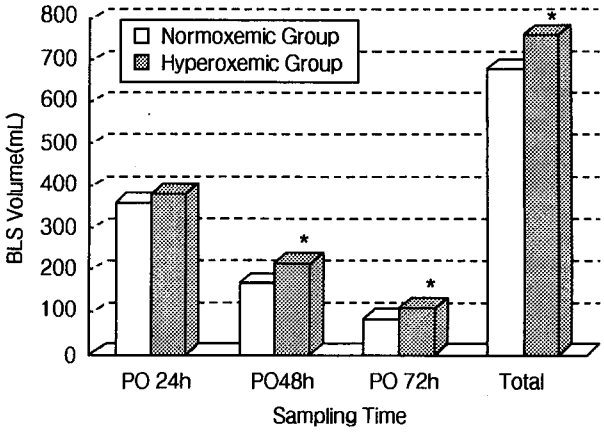

Fig. 7. Postoperative blood loss volume in normoxemic and hyperoxemic group. Blood loss at postoperative (PO) 48 hours, 72 hours and total periods were higher in hyperoxemic group compared to normoxemic group (*, $p<0.05$).

preoperative period (normoxemic=0.01±0.0 ng/mL vs hyperoxemic=0.01±0.0 ng/mL). Postoperative value in hyperoxemic group was significantly higher than that in normoxemic group ($p<0.05$) (Fig. 5).

6. Pulmonary vascular resistance

As shown in Fig. 6, pulmonary vascular resistance (PVR) was significantly reduced after operation in both groups ($p<0.05$). Postoperative value (113.80±13.15 dyne/sec/cm^5) in hyperoxemic group was significantly higher than that (91.73±15.68 dyne/sec/cm^5) in normoxemic group ($p<0.05$).

7. Postoperative blood loss

There was no significant difference between groups at postoperative 24 hours (normoxemic=360±67 mL vs hyperoxemic=380±71 mL) ($p>0.05$). However, blood loss volumes at postoperative 48 h, 72 h, and total periods were higher in hyperoxemic group compared with normoxemic group ($p<0.05$) (Fig. 7).

8. Biochemical markers

Biochemical markers are displayed in Table 2. Normoxemic group had elevated glucose and AST, while hyperoxemic group had increased iron, glucose, AST, ALT and BUN at postoperative period ($p<0.05$). All biochemical markers in hyperoxemic group were postoperatively higher than those in normoxemic group ($p<0.05$).

Table 2. Comparison of the biochemical markers between normoxemic and hyperoxemic group

Group / Parameter	Preoperative period		Postoperative period	
	Normoxemic	Hyperoxemic	Normoxemic	Hyperoxemic
Iron (μg/dL)	104.30±11.57	113.80±15.58	110.10±9.08	152.60±10.53^{+*}
Glucose (mg/dL)	95.35±5.87	93.60±4.20	134.0±10.43^{+}	180.73±10.43^{+*}
AST (IU/U)	23.50±6.32	22.74±4.05	59.36±9.46^{+}	91.0±11.28^{+*}
ALT (IU/L)	29.40±5.87	35.60±7.30	23.21±4.27	42.0±9.38^{+*}
BUN (mg/dL)	13.50±2.17	12.45±1.84	15.64±30.6	18.07±6.35^{+*}
CREAT (mg;dL)	0.85±0.05	0.94±0.06	0.88±0.05	1.02±0.06*

Data were expressed as mean ± standard error (SE).
+, $p<0.05$ (compared with preoperative period); *, $p<0.05$ (compared with normoxemic group).
Legend: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CREAT, creatinine

DISCUSSION

Extracorporeal circulation (ECC) for cardiovascular operations may produce reactive oxygen intermediates, oxygen free radicals, by altering complement system, neutrophil and arachidonic metabolism[8,14,25]. Also, higher partial pressure of arterial oxygen during ECC causes more production of oxygen free radicals[36]. Oxygen free radicals are highly cytotoxic reduced oxygen species(O_2^-·, H_2O_2 and OH·) and play a central role in oxygen-mediated injury[22]. However, hyperoxemic ECC has been widely used in most of cardiac centers. Belboul and colleagues showed that hyperoxemic ECC led to signifinicant increases in period of postoperative ventilator support, bleeding, blood product usage, arrhythmias, myocardial infarctions, respiratory insufficiency, liver enzymes, creatinine levels, and morbidity compared with normoxemic ECC. The postulation that oxygen-derived free radicals can be avoided by lowering PaO_2 during ECC is based on that the production of toxic oxygen species is proportionate to PaO_2[5,24,41]. Hyperoxemia sharply increases the amount of oxygen dissolved in the membrane lipid matrix, thereby enhancing the possibility of oxygen interaction with reduced electron carriers and accentuating free radical production[5]. According to an experimental animal model without ECC by Hearse and associates, myocardial damage following reoxygenation was highly dependent on PaO_2 and lowering oxyen levels resulted in reduced creatine kinase release[31]. Some studies suggested that hyperoxemia may induce myocardial oxidative damage for hypoxic immature heart, supporting the assumption that higher PaO_2 during ECC may be detrimental especially in cyanotic heart disease[34,35]. Furthermore, hyperoxemia may form gaseous vapor in perfusate, causing microemboli during ECC[46].

The present study confirms the deleterious effects of hyperoxemic ECC with more severe inflammatory reaction, increased postoperative bleeding, and elevated liver and kidney markers in hyperoxemic group compared with normoxemic group. Higher total leukocyte counts in hyperoxemic group are due to neutrophilia and considered an inflammatory marker. Neutrophils participate in pathophysiology related to ECC by various mechanisms. ECC induces neutrophil activation and activated neutrophils adhere to vascular endothelia of organs under ischemia/reperfusion, probably causing subsequent organ dysfunctions. NADPH (nicotinamide adenin dinucleotide phosphate) oxidase on the surface of neutrophil generates oxygen free radical[29,50]. Oxygen free radicals contribute to peroxidation of cellular membrane phospholipid and nucleic acid with sequent cell damage and destruction[51]. Neutrophils adhesions on vascular endothelium give rise to clumpping formation with secondary capillary obstruction and no-reflow phenomenon, which interrupts blood supply to distal areas of the organ[7,16]. Activated neutrophils-derived oxygen free radicals cause impairment of sarcoplasmic reticulum-induced cellular calcium transporting capacity and inhibiton of calcium-stimulated Mg^{2+}-dependent ATPase activity[6,16,54]. Na^+-Ca^{2+} exchange activity of cardiac sarcolemmal membrane is also influenced by free radicals[48]. In addition, activated neutrophils produce considerable amounts of cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-8 related to

development and progress of inflammatory reaction[17,18,47].

Activated neutrophils have deleterious effects not only on the heart but also the lung. When pulmonary circulation is reinstituted after cardiac operation, activated neutrophils adhere to pulmonary capillaries and are seqestered in pulmonary capillary beds, which subsequently release proteloytic enzymes to injure the pulmonary tissues[40,51].

The present data show postoperatively increased total leukocyte and neutrophil counts and higher PVR in hyperoxemic group compared with normoxemic group, suggesting that hyperoxemic ECC could lead to more severe inflammatory reaction and pulmonary injury than normoxemic ECC.

MDA, a marker of oxidative injury, is released with oxygen free radical-mediated lipid peroxidation of cellular membrane. Troponin T (TnT) is a myofibrillar structural protein of striated musculature localized within the thin filaments of the contractile apparatus[38]. This protein is cardiospecific with a molecular weight of 39 kDa (kilodalton) and a unique cardiac antigen which is continuously released from infarcting myocardium. The cardiospecificity of TnT might be particularly useful in assessing myocardial cell damage in patients underwent cardiac surgery[39]. The current study exhibits postoperatively higher MDA and TnT in hyperoxemic group, suggesting that hyperoxemic ECC generates more severe ischemia/reperfusion myocardial damages than normoxemic ECC.

Complex processes and mechanisms are involved in production of oxygen free radicals and subsequent tissue injury during ECC. In most of cardiac surgery, coronary and pulmonary circulations are interrupted and the heart and lung suffer ischemia for some periods. The metabolic disarrangements that occur during ischemia might predispose for the formation of free radicals from the residual molecular oxygen. There are many potential sources of free radicals in the myocardium. During ischemia the adenine nucleotide pool is partially degraded, thus leaving the mitochondrial carriers in a more fully reduced state[21,43]. This condition will result in a higher increase of electron leakage from the respiratory chain that, in turn, will react with the residual molecular oxygen entrapped with in the inner mitochondrial membrane, thus causing the formation of superoxide radicals. The increase of plasma hypoxanthine during hypoxia and/or ischemia could support the production of superoxide by xanthine oxidase during reoxygenation and/or reperfusion[52]. The other potential extracellular sources of oxygen free radicals are activated neutrophils. They possess a membrane-bound NADPH oxidase which produces superoxide. These activated neutrophils would begin to injure the tissue further by release of additional oxidative enzymes (myeloperoxidase) and hydrolytic enzymes such as elastase, collagenase, cathepsins, hyaluronidase[43]. In addition, the phenomenon of neutrophils plugging capillary beds will further reduce the circulation of blood, exacerbating ischemia[15,53]. Arachidonic acid is liberated and subsequently metabolized to prostaglandins and leukotrienes during ischemia[9,10,33]. These metabolic pathways involve electron transfers that can initiate the formation of free radicals[12,28]. Finally, the autooxidation of catecholamines, which are abundantly released from the ischemic myocardium, could provide through the formation of adrenochrome oxygen free radicals[3,57]. Furthermore, ischemia is associated with a depletion of the tissue content of antioxidants, including SOD, catalase, peroxidase, and GSH (glutathione peroxidase)[20,30,37,44,49]. Because free radical produciton is largely dependent on oxygen tension[23], it is expected that this phenomenon occurs to a much greater extent during reperfusion, when reactive hyperemia supplies abundant amounts of oxygen to previously "primed" sources of free radicals, than during ischemia. Restoration of coronary circulation also brings an additional source of free radicals consisting of activated polymorphonuclear leukocytes[42]. This burst of free radical generation overwhelms the scavenging capacity of the reduced cellular defense mechanism. As a result of this unbalance, free radical-mediated deleterious effects account for loss of normal mitochondrial and sarcoplasmic reticulum function, disturbed membrane permeability, and disruption of cellular transport processes, all of which a salient features of the so-called reperfusion injury[27,32,44]. In addition to lipid peroxidation, radical species are capable of various toxic effects such as inactivation of enzymes by oxidation of sulphydryl bounds, cross-linking of protein and DNA breakdown[19,55,56].

This study shows postoperatively elevated iron level in hyperoxemic group compared with normoxemic group. This finding contains an important implication because iron is associated with Haber-Weiss reaction which generates hydroxyl radical (OH·). Hydroxyl radical production actually results from a reaction between the one-electron reduced product superoxide anion (O_2^-·) and two-electron

reduced product hydrogen peroxide (H_2O_2). Hydroxyl radical is usually considered one of the most cytotoxic free radicals because of its strong oxidizing properties, which can affect almost all kinds of biomolecules. Haber-Weiss reaction requires the participation of a transitional metals catalyst, usually iron[26]. Iron is also involved in the chain reaction of lipid peroxidation because transitional metals catalyze the composition of lipid peroxides into unstable intermediates, which contribute to disruption of cell membranes. Therefore, higher iron level caused by hyperoxemic ECC may exaggerate ischemia/reperfusion injury during cardiovascular surgery. It seems likely that oxygen-derived free radicals may cause hemolysis during ECC and simultaneously release iron from the heme ring[11]. This may be a reasonable mechanism that can explain higher iron level in hyperoxemic group.

This study demonstrates that hyperoxemic ECC results in the whole body damaging effects as hyperoxemic group had postoperatively higher leukocyte and neutrophil counts, MDA, TnT, iron, glucose, blood loss, and liver and kidney markers. Additionally, the present observations support the hypothesis that hyperoxemic ECC is apparently nonphysiologic condition and more harmful. In conclusion, since hyperoxemic ECC seemed to damage postoperative outcomes, a more physiologic oxygen tension strategy during extracorporeal circulation should be considered hereafter in order to reduce the inflammatory and detrimental effects, and to improve patient recovery.

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