Oxidative Insult Associated With Hyperoxic Cardiopulmonary Bypass in the Infantile Heart and Lung

Kiyozo Morita, M.D., Kai Ihnken, M.D.*, Gerald D. Buckberg, M.D.* and Louis J. Ignarro, Ph.D.**

Cardiopulmonary bypass (CPB) per se alters many factors simultaneously, including free radical generation, which suggests that conventional hyperoxic CPB may produce oxidative injury in the infantile heart and lung. This study tests the hypothesis that CPB provokes oxidative cardiopulmonary changes and pulmonary endothelial dysfunction in immature piglets that can be prevented by free radical scavengers. We studied 15 2- to 3-week-old piglets. Five served as a control without CPB. Ten piglets underwent 60 min of CPB with a membrane oxygenator (Sarns). In 5 of these 10, the bypass prime was supplemented with N-mercaptopropionylglycine (MPG: 80 mg/kg) plus catalase (50,000 U/kg), whereas the others were not treated. Pre- and post-bypass cardiopulmonary function was measured in terms of left ventricular end-systolic elastance [Ees] by a conductance catheter, the arterial/alveolar pO₂ ratio (aA ratio) and static lung compliance. Conjugated dienes (A233 nm/mg lipid) were measured to detect lipid peroxidation in heart and lung tissue, and myocardial antioxidant reserve capacity [malondialdehyde (MDA) production in cardiac tissue incubated with the oxidant t-butyl hydroperoxide (t-BHP)] was assessed to detect oxidative changes. Pulmonary vascular resistance (PVR) and transpulmonary nitric oxide (NO) production were measured to assess pulmonary endothelial injury. Myocardial antioxidant reserve capacity was significantly reduced after 60 min of CPB, compared to control animals (MDA 779±100 vs 470±30 nmol/g protein, p<0.05 at t-BHP 2.0 mmol/L), without evidence of lipid peroxidation or myocardial dysfunction. Pulmonary vascular resistance after CPB was dramatically increased (83±12 to 212±30, p<0.05) without any change in lung function. In parallel to pulmonary vasoconstriction, NO production was significantly decreased after CPB (from 8.8±1.4 to 2.5±0.5 mmol/min/kg, p<0.05). The addition of antioxidants (MPG+catalase) to the prime significantly improved myocardial antioxidant status (MDA: 604±30 vs 779±100 nmol/g protein, p<0.05) and pulmonary vascular resistance (114±29 vs 212±30, p<0.05 vs no-treatment group).

In conclusion, the present study confirms that 1) Cardiopulmonary bypass produces substantial oxidative stress in normal immature myocardium, as assessed by reduced antioxidant reserve capacity; 2) CPB impairs pulmonary endothelial function, characterized by NO production, resulting in pulmonary vasoconstriction; and 3) These deleterious effects can be prevented by the addition of antioxidants (MPG/catalase) to the pump prime.

(Ipn Circ J 1996; 60: 355—363)

Key words:
Cardiopulmonary bypass
Free radicals
Antioxidant
Pulmonary hypertension

(Received May 10, 1995; accepted July 17, 1995)
Department of Cardiovascular Surgery, Jikei University School of Medicine, Tokyo, Japan
*Departments of Cardiothoracic Surgery, and **Pharmacology, UCLA School of Medicine, Los Angeles, CA, USA
Mailing address: Kiyozo Morita, M.D., 3-19-18 Nishi-shimbashi Minato-ku 105, Tokyo, Japan

Japanese Circulation Journal  Vol.60, June 1996  355
Cardiopulmonary bypass (CPB) is used with increasing frequency in early infancy or newborns to correct congenital heart defects. However, the incidence of postoperative cardiopulmonary dysfunction is reportedly higher in younger patients than in adults, which contributes to morbidity and mortality despite successful anatomical correction of congenital defects. Conventional CPB is initiated in patients, including infants and newborns, at hyperoxic pO2 levels (ie, ~400 mmHg) without considering any deleterious effect of a high O2 tension. However, the pathophysiology of conventional CPB has not been fully determined in infantile heart and lung.

Cardiopulmonary bypass per se alters many factors simultaneously leading to oxygen free radicals and vasoactive-mediator generation, contributing to postbypass heart and lung complications. Clinical reports have shown that infants and children have a greater inflammation-like response with CPB than adults. In contrast, the endogenous antioxidative defense system in the infantile heart is reportedly weaker than that in adults, and is thus more susceptible to oxidative stress. In addition, recent studies have shown that endothelium (coronary pulmonary and other vascular endothelium) is the principal target region of oxygen free radicals from extracellular sources (ie, activated leukocytes), which leads to endothelium-dependent vasoconstriction.

We speculate that the oxidative stress caused by CPB per se in the infantile heart, lung and vascular endothelium may contribute to postbypass cardiopulmonary dysfunction and pulmonary vasoconstriction.

In this study, we tested the hypothesis that CPB per se provokes cardiopulmonary oxidative insults in immature piglets, characterized by a reduction in myocardial antioxidant reserve and pulmonary endothelial dysfunction, as assessed by endothelium-derived relaxing factor (EDRF) or nitric oxide (NO) production, that can be prevented by the addition of antioxidants to the CPB circuit.

MATERIALS AND METHODS

Fifteen 2- to 3-week-old Yorkshire Duroc piglets (4-6 kg) were premedicated (0.5 mg/kg diazepam, im), anesthetized (30 mg/kg pentobarbital, followed by 5 mg/kg per h, iv), and ventilated by a volume-limited respirator (Sarvo 900D, Siemens-Elema, Sweden) via tracheostomy. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No.80-23, revised 1978). The ductus arteriosus was ligated routinely with a surgical clip via a left 4th intercostal thoracotomy. The heart was exposed by median sternotomy, and transducer-tipped catheters (Millar) were placed in the left ventricle, thoracic aorta, left atrium, and right atrium. A fluid-filled catheter connected to an external transducer was placed into the pulmonary artery. These signals were routed to a recorder (MT 9,000, Astro-Med Inc, West Warwick, RI) via signal conditioners (model 13-4615, Gould Inc, Eastlake, OH). A thermocoupling probe was directed into the main pulmonary artery and connected to a cardiac output computer (Model 9520A, American Edwards Laboratory, Santa Ana, CA).

Arterial blood gases, electrolyte and hemoglobin were measured (Blood Gas System 288, CIBA-Corning, Medfield, MA) to ensure optimal extracorporeal circulation. A heating pad maintained the monitored rectal temperature at 38°C.

An 8-electrode conductance catheter (interelectrode distance of 0.4 cm, Webster Laboratories, Baldwin Park, CA) was inserted through the left ventricular, apex and connected to a Sigma-5-DF signal conditioner-processor (Leycom, Oegstgeest, Netherlands).

After systemic heparinization (3 mg/kg iv) a single-stage venous cannula (20F) and an aortic cannula (8F) were inserted into the right atrial appendage and the left subclavian artery. The extracorporeal circuit was primed with packed red blood cells from donor pigs, with Ca2+ added to counteract

Japanese Circulation Journal Vol.60, June 1996
the citrate, hetastarch (Hespan DuPont, Wilmington, DE) and Plasma-Lyte electrolyte solution (Baxter Healthcare, Deerfield, IL). During perfusion, hematocrit was maintained at 25–35%. A membrane oxygenator (Sarns 16310 Membrane Oxygenator, Sarns, Ann Arbor, MI) was used. During extracorporeal circulation, arterial pO₂ and perfusion flow were maintained between 400–500 mmHg and at 100 ml/min/kg, respectively.

**Experimental Groups**

**Control group:** Five piglets were anesthetized, instrumented, and observed over 5 h, to provide baseline control functional and biochemical data.

**CPB group:** Ten piglets underwent 60 min of CPB with Sarns membrane oxygenators, followed by 60 min of observation after the discontinuation of CPB. In 5 piglets, the bypass prime was supplemented with a) N-mercaptopropionylglycine (MPG: 80 mg/kg) plus catalase (50,000 U/kg), whereas the other 5 piglets were not treated.

**Evaluations**

**A. Cardiac:**

1. **Left ventricular contractility:**

   Left ventricular (LV) pressure and conductance catheter signals were amplified and digitized to inscribe left ventricular pressure-volume loops. After the correction of parallel conductance, a series of pressure-volume loops under variable loading conditions was generated by rapid transient occlusion of the inferior vena cava during 7 sec of apnea, at a under control conditions and 30 min after discontinuing CPB.

   The end-systolic pressure-volume relationship (ESPVR) was analyzed using the interactive video graphics program “Spectrum” on a 383/33 MHz IBM PC, and LV performance was described in terms of the slope of the linear regression, (Ees: End-systolic Elastance), as previously reported.

2. **Myocardial tissue injury due to oxidants:**

   a) Antioxidant reserve capacity: To assess the myocardial antioxidant state, in vitro lipid peroxidation was measured as a function of the concentration of the oxidant t-butyl hydroperoxide (t-BHP), as described by Godin et al. Myocardial tissues were homogenized and incubated with 0–4 mmol/L of t-butyl hydroperoxide for 30 min at 37°C. The formation of TBA-reactive substances, an index of lipid peroxidation, was measured spectrophotometrically at 532 nm, and a reaction curve was generated. A malondialdehyde (MDA) standard curve was generated simultaneously, and lipid peroxidation was expressed as nmol MDA/g protein.

   b) Conjugated dienes in myocardial tissue were measured as a marker of lipid peroxidation. Endocardial biopsy specimens were immediately frozen and stored in liquid nitrogen, and tissue levels of hydroxyconjugated dienes were determined after chloroform: methanol (2:1, v/v) extraction as described by Lesnfsky et al. The content of conjugated dienes was expressed in terms of the absorbance at 233 nm (A₂₃₃ nm/mg lipid).

**B. Pulmonary Circulation:**

1. **Pulmonary vascular resistance:**

   Cardiac output was determined by duplicate injections of 1 ml of 4°C saline into a central venous catheter, under control conditions and 30 min after discontinuing CPB. Pulmonary vascular resistance index (PVRI) was calculated as

   \[
   \text{PVRI} = \frac{(\text{PAP} - \text{LAP})/\text{CO}}{\text{mmHg/L/min}} \times \text{Body Weight [kg]}
   \]

   where PAP is mean pulmonary artery pressure, LAP is left atrial pressure, and CO is cardiac output in liters per minute.

2. **Nitric oxide (NO) plasma concentration and pulmonary nitric oxide production:**

   The nitric oxide concentration was determined in pulmonary artery and vein plasma in terms of its spontaneous oxidation product, nitrite (NO₂⁻), which was recombined to NO and quantitated with a sensitive chemiluminescence assay using a nitrogen oxide analyzer (DASIBI Environmental Corp, Model 2108, NOx analyzer, Glendale, CA). The method was modified to increase the sensitivity of the detector to 0.8 ppb of nitric oxide (1 pmol/0.1 ml of test sample). Plasma samples were obtained under control conditions and at 30 and 60 min after discontinuing CPB. Pulmonary NO production
### TABLE I MYOCARDIAL FUNCTION AND BIOCHEMICAL CHANGES AFTER CPB

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CPB</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV %Ees</td>
<td>105±5</td>
<td>97±4</td>
<td>NS</td>
</tr>
<tr>
<td>Conjugated Dienes (A233 nm/mg lipid)</td>
<td>0.71±0.06</td>
<td>0.69±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Antioxidant Reserve MDA (nmol/g protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At-BHP: 1.0 mmol/L</td>
<td>241±24</td>
<td>383±44</td>
<td>0.012</td>
</tr>
<tr>
<td>At-BHP: 2.0 mmol/L</td>
<td>471±30</td>
<td>779±99</td>
<td>0.004</td>
</tr>
</tbody>
</table>

### TABLE II PULMONARY FUNCTION AND BIOCHEMICAL CHANGES AFTER CPB

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CPB</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVRI(dyn sec cm⁻⁵ kg)</td>
<td>83±12</td>
<td>212±30</td>
<td>0.0002</td>
</tr>
<tr>
<td>Lung Function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/A pO₂ Ratio</td>
<td>0.66±0.04</td>
<td>0.62±0.11</td>
<td>NS</td>
</tr>
<tr>
<td>% of Static Compliance</td>
<td>104.9±12</td>
<td>86.6±16</td>
<td>NS</td>
</tr>
<tr>
<td>Conjugated Dienes (A233 nm/mg lipid)</td>
<td>0.618±0.03</td>
<td>0.707±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Tissue water content(%)</td>
<td>82.3±0.67</td>
<td>81.2±0.78</td>
<td>NS</td>
</tr>
</tbody>
</table>

was calculated as

\[
\text{NO production (\(\mu\text{mol/min/kg}) = (NO_{LA} - NO_{PA}) \times CO (ml/min)/Body Weight (kg)}
\]

where \(NO_{LA}\) = NO concentration in LA plasma, and \(NO_{PA}\) = NO concentration in PA plasma.

**C. Lung:**

1. **Intrapulmonary shunting:**

The ratio between arterial and alveolar oxygen partial pressure (a/A pO₂ ratio) was calculated as

\[
a/A \text{ ratio} = pA_{O_2}/pA^{O_2}
\]

where \(pA^{O_2} = (P_{atm} - pH_2O) FIO_2 - PA^{CO2}\). This ratio is relatively stable with a varying FIO₂, unlike the classic alveolar-arterial gradient. The normal a/A ratio is 0.75.

2. **Static lung compliance:**

Static lung compliance (LC) was determined from duplicate expirations using a Siemens 900 D ventilator. Expiratory plateau pressure was recorded from 2 breaths at 4 different tidal volumes (15, 30, 45 and 60 ml). LC was expressed as ml/cm H₂O and assessed in terms of % recovery after CPB.

3. **Lung tissue conjugated dienes and lung water content:**

After the functional assessment, lung biopsy specimens were immediately frozen and stored in liquid nitrogen, and tissue levels of hydroxyconjugated dienes were determined as described previously. The content of conjugated dienes was expressed in terms of absorbance at 233 nm (A₂₃₃ nm/mg lipid). Another lung specimen was taken to measure lung water content and expressed as % of wet weight.

**Statistical Analysis**

Data were analyzed with StatView V2.0 on an Apple Macintosh IIci. An analysis of variance (ANOVA) was used for comparisons between groups. Differences were considered significant at a probability level of \(p < 0.05\). Group data are expressed as mean ± SEM.

**RESULTS**

1. **Cardiac**

Myocardial antioxidant reserve capacity was significantly reduced after 60 min of
CPB, since malondialdehyde (MDA) production in myocardium subjected to CPB was greater than that in the control animals (Table I). In contrast, the myocardial conjugated diene level was comparable to that in the control piglets and no myocardial dysfunction was not observed.

2. Pulmonary

PVRI was dramatically increased after CPB (83±12 to 212±32, p<0.05) CPB caused a 15% increase in parenchymal lipid peroxidation as assessed by conjugated dienes from lung homogenates, but this increase was not statistically significant. In addition, there was no evidence of changes in lung function as assessed by the a/A ratio or lung compliance (Table II).

3. Pulmonary nitric oxide production
Transpulmonary nitric oxide measurement suggested that the lung produced NO, since NO levels in the left atrium (pulmonary vein) were always slightly greater than those in the pulmonary artery (Fig 1). This transpulmonary gradient was maintained post-CPB, but absolute NO content progressively decreased. Consequently, transpulmonary NO production [cardiac output × NO in (LA−PA)] was reduced 73% by 60 min of CPB, in parallel to pulmonary vasoconstriction (Fig 2).

4. Effects of free radical scavengers

The addition of MPG (80 mg/kg) plus catalase to the pump prime produced a substantial improvement of myocardial antioxidant status and was associated with nearly normal pulmonary vascular resistance after CPB (Figs 3, 4).

DISCUSSION

The present study confirms that 1) routine CPB causes substantial oxidative stress to normal immature myocardium, 2) CPB impairs pulmonary endothelial function, characterized by endothelium-derived relaxing factor (EDRF), or nitric oxide (NO) production, resulting in postbypass pulmonary vasoconstriction without significant alterations in lung function, and 3) these deleterious effects of CPB in immature piglets may be mediated by free radical generation, which can be prevented by the addition of antioxidants (free radical scavengers) to the pump prime. These findings clearly demonstrate the oxidative potential of conventional hypoxic CPB in infantile hearts and pulmonary endothelium, which may contribute to postbypass cardiopulmonary complications in pediatric open-heart operations.

Complement-related neutrophil activation and subsequent production of oxygen free radicals due to cardiopulmonary bypass were thought to be responsible for postbypass cardiac dysfunction and lung complications. Clinical reports have shown that infants and children exhibit a greater inflammation-like response mediated by complement activation with CPB than adults. In contrast, the antioxidative defense profile in younger patients are reportedly lower than those in adults. However, little information is available regarding the effects of oxidative myocardial changes in immature hearts during conventional hypoxic CPB. In the present study, antioxidant reserve capacity was measured in terms of in vitro lipid peroxidation to evaluate the myocardial oxidative status. Godin et al have shown that this measurement reflects the status of endogenous defense mechanisms (i.e., glutathione peroxidase, reduced glutathione). When tissue is subjected to oxidative stress, endogenous superoxide dismutase (SOD) catalyzes dismutation of generated superoxide anion to hydrogen peroxide, which decays further to a highly toxic oxygen species, OH, via the traditional Haber-Weiss reaction. The glutathione peroxidase (GPD)-oxidized glutathione reductase system, which requires reduced glutathione (GSH) as an electron substrate, is the primary enzymatic scavenger for hydrogen peroxide. Furthermore, hydroxyl radicals, produced via the Haber-Weiss or an alternate pathway from the interaction between O₂⁻ and nitric oxide (NO), can be removed by an intracellular thiol compound (eg, GSH). Thus, prolonged oxidative stress can deplete these endogenous antioxidants. The present study showed that CPB per se caused substantial depletion of myocardial endogenous antioxidants, which can be restored by adding antioxidants (MPG plus catalase) to the CPB prime, signifying that CPB per se generates oxygen radicals. However, normal myocardium can overcome such oxidative stress, by virtue of an endogenous antioxidant reserve capacity, to prevent lipid peroxidation and subsequent myocardial dysfunction. In contrast, the oxidative myocardial changes due to CPB are particularly important when considering previously damaged myocardium (ie, preoperative cardiac shock, hemodynamic instability, ischemia) or chronic cyanosis, in which endogenous antioxidants may have already been depleted and which may be easily overwhelmed by oxidative stress due to CPB per se, leading to lipid peroxidation and myocardial dysfunction.

Pulmonary dysfunction after CPB has long been a major problem in cardiac operations, and recent studies have revealed that complement and leukocyte activation plays a role in its pathogenesis. Bando et al have shown in adult dogs that CPB with bubble
Oxidative Changes by Cardiopulmonary Bypass

Oxygenators caused profound lung dysfunction, associated with an increased level of plasma conjugated dienes, lung edema and pulmonary vasoconstriction. Conversely, in the present study, we observed relatively normal lung function as assessed by static lung compliance and the a/A \( P_{O_2} \) ratio, with a slight increase in lipid peroxidation. This discrepancy may be due to the use of a membrane oxygenator and species differences.

Pulmonary vasoconstriction has been demonstrated after CPB, both experimentally and clinically. Some reports imply that a systemic inflammation-like reaction including free radicals from activated neutrophil and thromboxane \( A_2 \) may play a role in its pathogenesis. More recently it has been shown experimentally that total CPB, which may cause complete cessation and reestablishment of pulmonary artery flow, produces ischemia-reperfusion lung injury, characterized by alveolar epithelial cells and capillary endothelial cell injury, possibly due to local activation of the complement leukocyte system, leading to free radical generation. Vascular endothelium plays an important role in regulating vascular tone by releasing vasoactive mediators, including endothelium-derived relaxing factor (EDRF) and prostacyclin. It is well established that endothelium (coronary\(^9\), pulmonary\(^12\) and other vascular endothelium\(^13,14\)) is the initial target region of free radical attack from extracellular sources (ie, activated leukocytes), resulting in endothelium-dependent vasoconstriction. Ohlstein and Nichols\(^14\) have shown in an in vitro study that activated neutrophil, which is associated with CPB, causes endothelium-dependent vasoconstriction in normal rabbit aortic segments. In the present study, EDRF or nitric oxide was determined in plasma as its oxidation product, nitrite (\( NO_2^- \)), because of its short half-life. Since the biological reaction (inactivation) of NO can be accounted for by spontaneous oxidation of NO to \( NO_2^- \), measurement of nitrite allows us to determine the NO concentration. Based on the results of this measurement, we confirmed in vivo that CPB per se produces pulmonary vasoconstriction in parallel to pulmonary endothelial dysfunction as assessed by pulmonary NO production, and that this is not necessarily associated with lung tissue injury. These findings might facilitate the use of inhaled NO for pulmonary hypertension after pediatric open-heart surgery.

It has been well documented\(^11,29\) that \( O_2^- \) is responsible for inactivating EDRF. On the other hand, hydrogen peroxide and subsequent production of hydroxyl radical appears to be involved in more profound endothelium damage and morphological disruption.\(^30\) Recently, Marczin et al\(^12\) showed, using cocultures of calf pulmonary artery endothelial cells and rabbit pulmonary artery smooth muscle cells, that brief exposure to \( H_2O_2 \) causes a dose-dependent impairment of pulmonary endothelial function (EDRF release), as assessed by cGMP formation in smooth muscles. They further suggested that hydrogen peroxide and subsequent iron-catalyzed hydroxyl radical formation may inhibit EDRF synthesis. Our findings which demonstrate the protective effects of MPG (hydroxyl radical scavenger) plus catalase (scavenger for hydrogen peroxide) are consistent with their findings, and strongly suggest that these oxidant species are responsible for the pathogenesis of postbypass pulmonary vasoconstriction.

Based on these findings, we conclude that 1) Cardiopulmonary bypass causes a substantial oxidative stress to normal immature myocardium, as assessed by a reduced antioxidant reserve capacity; 2) This myocardial oxidative challenge is not associated with lipid peroxidation or functional deterioration; 3) CPB impairs pulmonary endothelial function, characterized by endothelium-derived relaxing factor (EDRF), or nitric oxide (NO) production, resulting in postbypass pulmonary vasoconstriction; and 4) These deleterious effects of CPB in immature piglets may be mediated by free radical generation which can be prevented by the addition of antioxidants (MPG/catalase) to the pump prime.

Acknowledgments

The authors wish to thank to Garland Hodges, Russell Byrns, and Nanci Stellino for their technical assistance, and Judith Becker for help in preparing the manuscript.

This research was supported by grants from the National Heart, Lung, and Blood Institute (HL-40675 and HL-40922) and the University of California Tobacco-Related Disease Research program.

Japanese Circulation Journal Vol.60, June 1996
REFERENCES


25. Latson TW, Krickler TS, Baumgartner WA: Pulmonary hypertension and noncardiogenic pulmonary edema following cardiopulmonary bypass associated with an antigenylantibody antibody. Anesthesiology 1986; 64: 106–111


27. Ignarro LJ: Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev
