Does Normoxemic Cardiopulmonary Bypass Prevent Myocardial Reoxygenation Injury in Cyanotic Children?

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Objective: **To evaluate whether the deleterious effect of cardiopulmonary bypass (CPB) can be prevented by controlling PaO_2 in cyanotic children.**

Design: **Prospective, randomized, clinical study.**

Setting: **Single university hospital.**

Participants: **Pediatric patients undergoing cardiac surgery for repair of congenital heart disease (n = 24).**

Interventions: **Patients were randomly allocated into 3 groups. Patients in the acyanotic group (group I, n = 10) had CPB initiated at a fraction of inspired oxygen (F_IO_2) of 1.0 (PO_2, 300 to 350 mmHg). Cyanotic patients were subdivided as follows: Group II (n = 7) had CPB initiated at an F_IO_2 of 1.0, and group III (n = 7) had CPB initiated at an F_IO_2 of 0.21 (PO_2, 90 to 110 mmHg). A biopsy specimen of right atrial tissue was removed during venous cannulation, and another sample was removed after CPB before aortic cross-clamping. The tissue was incubated in 4 mmol/L of t-butylhydroperoxide, and the malondialdehyde (MDA) level was measured to determine the antioxidant reserve capacity. Blood samples for cytokine levels, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 response to CPB were collected after induction of anesthesia and at the end of CPB before protamine administration.**

Measurements and Main Results: **After initiation of CPB, MDA level rose markedly in the cyanotic groups compared with the acyanotic group (210 ± 118% *v* 52 ± 34%, $p < 0.05$), which indicated the depletion of antioxidants. After initiation of CPB, TNF-α and IL-6 levels of the cyanotic groups were higher than for the acyanotic group (168 ± 77 *v* 85 ± 57, $p < 0.001$; 249 ± 131 *v* 52 ± 40; $p < 0.001$). When a comparison between the cyanotic groups was performed, group II (initiating CPB at an F_IO_2 of 1.0) had significantly increased MDA production compared with group III (initiating CPB at an F_IO_2 of 0.21) (302 ± 134% *v* 133 ± 74%, $p < 0.05$). Group II had higher TNF-α and IL-6 levels than group III (204 ± 81 *v* 131 ± 52, $p < 0.001$; 308 ± 147 *v* 191 ± 81, $p < 0.01$).**

Conclusion: **Conventional clinical methods of initiating CPB at a hyperoxemic PO_2 may increase the possibility of myocardial reoxygenation injury in cyanotic children. This deleterious effect of reoxygenation can be modified by initiating CPB at a lower level of oxygen concentration. Subsequent long-term studies are needed to determine the best method of decreasing the oxygen concentration of the CPB circuit.**

KEY WORDS: reoxygenation injury, antioxidant reserve capacity, cyanotic congenital heart, cardiopulmonary bypass (CPB)

DESPITE SUCCESSFUL REPAIR of congenital heart defects causing cyanosis in infancy and early childhood, postoperative myocardial dysfunction remains a more common cause of morbidity and mortality compared with repair of acquired defects in adults with normoxic conditions.[1] Experimental and clinical studies indicate that this phenomenon is partly the result of reoxygenation injury that occurs with the onset of a high oxygen supply during cardiopulmonary bypass (CPB) leading to free radical production and lipid peroxidation.[2-4] Additionally, CPB induces complex inflammatory mechanisms, including the synthesis of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, which may be related in part to postoperative complications.[5,6] This study investigates whether the conventional clinical methods of initiating CPB at a hyperoxemic PaO_2 produces a reoxygenation injury in cyanotic children and if this deleterious effect can be modified by initiating CPB at a lower level of oxygen concentration.

PATIENTS AND METHODS

After receiving approval by the ethics committee and informed consent, the authors studied 24 children undergoing CPB for congenital heart defect repair. Patients, ranging in age from 3 months to 5 years, were randomly allocated into 3 groups. Patients undergoing reoperations, patients undergoing deep hypothermia, and patients with hemodynamic and clinical signs of low cardiac output were excluded from the study.

Anesthesia was induced with fentanyl, 25 μg/kg; vecuronium, 0.1 mg/kg; midazolam, 0.1 mg/kg; and an oxygen-air mixture. Mechanical ventilation was provided by a Servo 900 C (Siemens-Elema, Solna, Sweden) during the operation. Volume ventilation was adjusted to maintain $PaCO_2$ between 30 and 35 mmHg. In all patients, a median sternotomy was performed. CPB was initiated after standard aortobicaval cannulation. A membrane oxygenator (Minimax Plus, Medtronic Inc, Anaheim, CA) and a nonpulsatile roller pump (Model 10.10.00; Stöckert Instruments, Munich, Germany) were used. Priming fluids consisted of isotonic sodium chloride supplemented with heparin; mannitol, 0.5 mg/kg; and aprotinin, 30,000 KIU/kg. Fresh whole blood was added to the priming solution in appropriate amounts to achieve a hematocrit of 20% to 22% during CPB. Pump flows were 2.4 to 2.6 L/min/m^2 during the normothermic period. Blood cardioplegia with 25 mEq/L of potassium (30 mL/kg for induction) was injected into the aortic root and repeated every 20 minutes. Acid-base status and blood gases were adjusted according to the α-stat method during CPB. Moderate hypothermia (26°C to 28°C) was used during CPB. Dopamine infusions were kept to a rate <10 μg/kg/min, and the occasional patients who required additional inotropic support received an epinephrine infusion in a dose <0.05 μg/kg/min.

Ten children who had a normal oxygen saturation were considered to be acyanotic (group I); CPB was initiated at a fraction of inspired oxygen (F_IO_2) of 1.0 (prime was circulated using 100% oxygen [PaO_2, 300 to 350 mmHg]). Fourteen children who had an oxygen saturation of $<85\%$ were considered to be cyanotic; in 7 of these children (group II), the CPB was initiated at an F_IO_2 of 1.0. In the other 7 cyanotic children (group III), CPB was initiated at an F_IO_2 of 0.21 (pump prime was circulated using 21% oxygen, resulting in a PaO_2 of 90 to 110 mmHg).

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Table 1. Demographic Characterictics of Patients

	Group I (n = 10)	Group II (n = 7)	Group III (n = 7)
Age (mo)	21 ± 17	26 ± 15	24 ± 11
Sex (M/F)	5/5	4/3	4/3
Weight (kg)	8.75 ± 2.68	9.11 ± 2.45	11.16 ± 2.64
Cross-clamp time (min)	62 ± 15	72 ± 17	75 ± 21
Duration of CPB (min)	98 ± 12	115 ± 24	118 ± 22
Intubation period (hr)	28.6 ± 28.9*	42.3 ± 40.1	45.6 ± 43.5
Stay in ICU (hr)	85 ± 40.6*	115.4 ± 110.5	122 ± 118.1

NOTE. Values are expressed as mean ± SD. No statistical difference was found among the groups in any of the variables except the intubation period and stay in the intensive care unit (ICU).

*$p < 0.05$.

A sample of right atrial tissue was removed in all patients during venous cannulation, and another sample was removed 10 to 15 minutes after initiating CPB and before aortic cross-clamping. The myocardial antioxidant reserve capacity was assessed according to the method of Godin et al[7] by determining *in vitro* lipid peroxidation in cardiac tissue. Briefly, the tissue was incubated with t-butylhydroperoxide at a concentration of 4 mmol/L for 15 minutes at 37°C. Lipid peroxidation was determined by measuring thiobarbituric acid–reactive substances spectrophotometrically at 532 nm. Lipid peroxidation is expressed as nanomoles malondialdehyde (MDA) per gram protein of tissue. The antioxidant reserve capacity is expressed as the percentage increase in MDA production compared with post-CPB levels in cyanotic groups.

To determine the synthesis of proinflammatory cytokines, such as TNF-α and IL-6, blood samples were collected from all patients after induction of anesthesia and at the end of CPB before protamine administration. Blood was immediately centrifuged (3,000 rpm for 10 minutes), and separated plasma was frozen at −70°C until assay. TNF-α and IL-6 were determined by means of a photometric enzyme-linked immunosorbent assay (human TNF-α and human IL-6 pg/mL; Boehringer, Mannheim, Germany).

Data were analyzed, and paired Student *t*-test was used for comparing variables among groups at a probability level of < 0.05. Group data are expressed as the mean ± SD.

RESULTS

The average age of patients was 24 ± 18 months (range, 3 months to 5 years). The demographic characteristics and pathologic properties of the groups are shown in Tables 1 and 2. There was no difference in the demographics among the 3 groups except for the shorter duration of intubation and the stay in the intensive care unit in the acyanotic group.

Results of measurements are summarized in Figs 1 to 5. There was no difference in the pre-CPB MDA levels between cyanotic and acyanotic groups (352 ± 62 nmol MDA/g protein *v* 300 ± 34 nmol MDA/g protein). After CPB, the MDA level rose markedly in the cyanotic groups compared with the acyanotic group (1,091.2 ± 118 nmol MDA/g protein *v* 456 ± 34 nmol MDA/g protein; $p < 0.05$), which indicated the depletion of antioxidants (Fig 1). When basal serum levels of TNF-α were compared, there were no significant differences between the cyanotic and acyanotic groups (20 ± 5 pg/mL *v* 18 ± 4 pg/mL). After initiation of CPB, TNF-α levels of the cyanotic groups were higher than the acyanotic group (168 ± 77 pg/mL *v* 85 ± 57 pg/mL, $p < 0.001$) (Fig 2). Pre-CPB levels of IL-6 had no difference between cyanotic and acyanotic groups (17 ± 2 pg/mL *v* 16 ± 2 pg/mL). After initiation of CPB, IL-6 levels of the cyanotic groups were higher than the acyanotic group (249 ± 131 pg/mL *v* 52 ± 40 pg/mL, $p < 0.001$) (Fig 3). When the cyanotic groups were compared, group II (initiating CPB at an F_IO_2 of 1.0) had significantly increased MDA production versus group III (initiating CPB at an F_IO_2 of 0.21) (302 ± 134% *v* 133 ± 74%, $p < 0.05$) (Fig 4). When serum TNF-α and IL-6 levels were compared between the cyanotic groups, group II had higher TNF-α (204 ± 81 pg/mL *v* 131 ± 52 pg/mL, $p < 0.01$) and higher IL-6 levels than group III (308 ± 147 pg/mL *v* 191 ± 81 pg/mL, $p < 0.01$) (Fig 5).

Table 2. Pathology of Groups

Pathology	Group I (n = 10), Acyanotic	Group II (n = 7), Cyanotic	Group III (n = 7), Cyanotic
ASD	3		
VSD	5		
VSD + Aort coarct	2		
TOF		3	2
TOF + PA			2
DORV		1	1
DORV + PS		1	1
TA		2	
TA + VSD			1

Abbreviations: ASD, atrial septal defect; VSD, ventricular septal defect, Aort coarct, coarctation of aorta; TOF, tetralogy of Fallot; PA, pulmonary atresia; DORV, double-outlet right ventricle; PS, pulmonary stenosis; TA, tricuspid atresia.

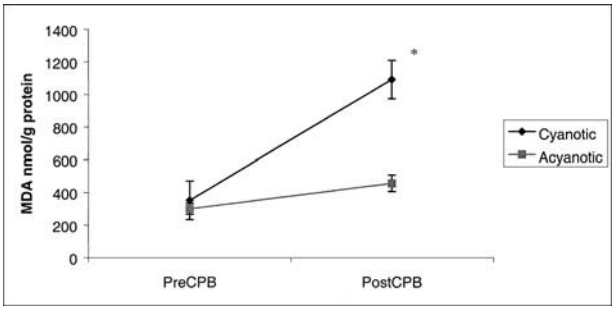

Fig 1. Antioxidant reserve capacity in cyanotic and acyanotic groups before and after CPB. There is a significant loss of the antioxidant reserve capacity in the cyanotic group, which indicates a great exposure to oxygen free radicals. *$p < 0.05$.

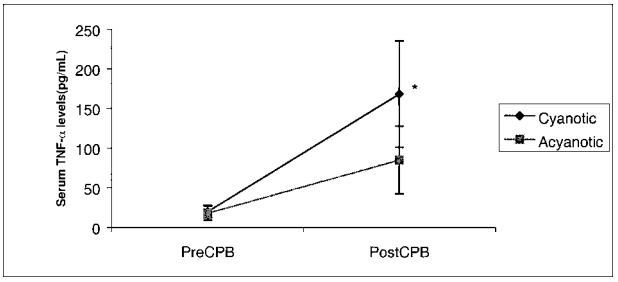

Fig 2. Serum TNF-α levels in cyanotic and acyanotic groups before and after CPB. *$p < 0.001$.

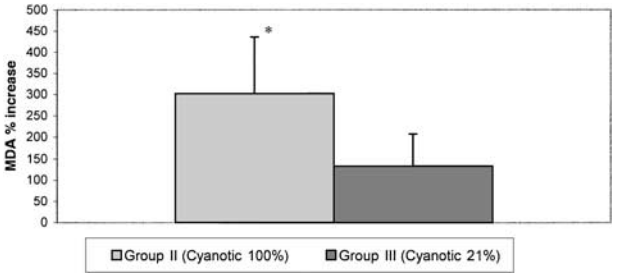

Fig 4. Increase in MDA production between cyanotic groups after CPB. Increase of MDA, in group II using an F_IO_2 of 1.0, which indicated the depletion of antioxidants. *$p < 0.05$.

DISCUSSION

Despite improvements in total correction of cyanotic heart defects, the low output syndrome is still the principal cause of morbidity and mortality. It is believed that peripheral tissue perfusion is disturbed during hypothermic CPB; the routine clinical procedure of initiating CPB with a PaO_2 of approximately 300 to 400 mmHg is used by priming the extracorporeal circuit with hyperoxemic fluid.[8] Studies indicate, however, that an unintended injury occurs in response to sudden reoxygenation of cyanotic hearts, and it is suggested that this phenomenon is partly the result of a reoxygenation injury leading to free radical production, lipid peroxidation, and impaired myocardial contractility.[9-12] The present study supports the validity of these experimental findings by showing that cyanotic children reoxygenated on CPB with a high F_IO_2 have a lower level of antioxidant reserve capacity. Several methods to evaluate the functional and biochemical effects of hypoxia and reoxygenation are available. The antioxidant reserve capacity is determined by adding a strong oxidant (t-butylhydroperoxide) to myocardial tissue and measures the tissues' ability to scavenge the resulting oxygen radicals and prevent MDA formation (a byproduct of lipid peroxidation). The more MDA produced, the lower the levels of these endogenous stores. Tissue oxidants are lost when oxygen free radicals are produced and need to be scavenged, such as when the hypoxemic heart is abruptly reoxygenated during initiation of CPB. The authors chose this test because it has been used in previous experimental studies of acute hypoxia and allows for comparison of the clinical results.

In the present study, myocardial tissue samples were removed before and after CPB was initiated to determine the antioxidant reserve capacity. There was no difference in the pre-CPB antioxidant reserve capacity between the cyanotic and acyanotic groups. Sudden reoxygenation of the cyanotic groups resulted in a significant depletion of antioxidants. Cyanotic children reoxygenated using an F_IO_2 of 1.0 (group II, PaO_2, 300 to 350 mmHg) showed the greatest loss of the myocardial antioxidant reserve capacity (highest MDA formation), indicating the greatest exposure to oxygen free radicals. Minimal change of antioxidant reserve capacity of the acyanotic group indicates that free oxygen radical production in the absence of hypoxemia is not present.

This oxidant damage in response to sudden reoxygenation has been previously studied in experimental models. These *in vivo* studies showed that reoxygenation after hypoxemia causes myocardial contractile dysfunction that is linked to biochemical evidence of lipid peroxidation and expenditure of endogenous antioxidant reserve capacity.[13,14] Ihnken et al[3] described reoxygenation injury associated with lipid peroxidation and decreased post-CPB contractility in cyanotic immature hearts when reoxygenated on CPB. In an isolated rat heart model, Schlüter et al[15] showed abrupt enzyme release on reoxygenation and provided ultrastructural evidence for a reoxygenation injury.

The present findings closely parallel the experimental studies; however, previous clinical studies are rare. Del Nido et al[16] showed a reoxygenation injury with subsequent lipid peroxidation during repair of tetralogy of Fallot. They indicated that the routine clinical procedure of starting CPB with a higher

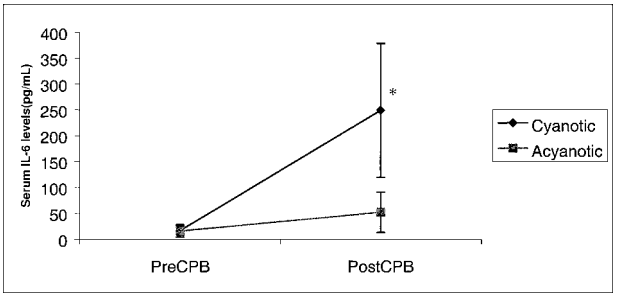

Fig 3. Serum IL-6 levels in cyanotic and acyanotic groups before and after CPB. *$p < 0.001$.

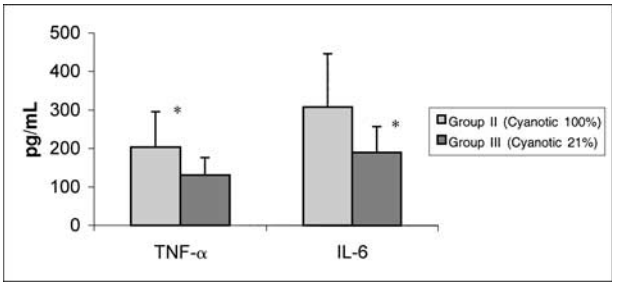

Fig 5. TNF-α and IL-6 levels of cyanotic groups after CPB. *$p < 0.01$.

PaO_2 in cyanotic infants may expose them to the damaging effect of a free radical–imposed reoxygenation injury, which may be followed by lipid peroxidation and reduced antioxidant reserve capacity. The present findings also confirm the results of Allen et al,[17] who suggested oxygen free radical production can be limited by decreasing the oxygen concentration of the CPB circuit or more effectively by leukocyte filtration in cyanotic children during CPB.

CPB induces complex inflammatory changes, including the release of proinflammatory cytokines, such as TNF-α and IL-6, which may be related in part to postoperative complications.[18] It is well known that these mediators of systemic inflammation in conjunction with ischemia-reperfusion injury account for widespread organ injury during and after CPB.[5,6] Seghaye et al[19] suggested that a rapid increase of TNF-α after removal of the aortic cross-clamp and the initiation of rewarming supports the view that rewarming and ischemia-reperfusion mechanisms could be responsible at least in part for TNF-α release. The authors have not found any study, however, that showed the cytokine levels related to different oxygen concentrations in cyanotic children at the start of CPB. In this study, after initiation of CPB, TNF-α and IL-6 levels of cyanotic groups were higher than the acyanotic group, confirming previous reports. Also, when the cyanotic groups were compared, group II initiated CPB with a higher oxygen concentration and had a higher level of cytokine release, increasing the possibility of injury.

In conclusion, this study showed that conventional clinical methods of initiating CPB at a higher level of oxygen concentration may increase the possibility of reoxygenation injury characterized by lipid peroxidation and cytokine release. This deleterious effect of sudden reoxygenation may be modified by initiating CPB at a lower level of oxygen concentration. Further long-term studies are needed to determine the effect of normoxemic CPB on the outcome of cyanotic congenital heart defects.

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