STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY: WITH AORTIC CLAMPING

XII. Delay of cardiac reoxygenation damage in the presence of cyanosis: A new concept of controlled cardiac reoxygenation

Twenty-one immature piglets (<3 weeks old) underwent 30 minutes of aortic clamping with hypocalemic glutamate/aspartate blood cardioplegia. Six piglets underwent hyperoxemic cardiopulmonary bypass and blood cardioplegia without preceding hypoxemia (control). Fifteen piglets became hypoxemic (oxygen tension about 25 mm Hg) for up to 2 hours by decreasing ventilator fraction of inspired oxygen to 6% to 7% before cardiopulmonary bypass. Of these, six piglets underwent 5 minutes of abrupt hyperoxemic uncontrolled reoxygenation by starting cardiopulmonary bypass at oxygen tension of about 400 mm Hg before they received oxygen tension of about 400 mm Hg blood cardioplegia. Nine others underwent controlled cardiac reoxygenation by starting cardiopulmonary bypass at ambient oxygen tension (about 25 mm Hg) followed 5 minutes later by 30 minutes of cardiopulmonary bypass at normoxemic oxygen tension (about 100 mm Hg) before raising oxygen tension to about 400 mm Hg. Myocardial function after cardiopulmonary bypass was evaluated from end-systolic elastance by conductance catheter, oxidant damage was estimated by measuring transcoronary conjugated diene levels to detect lipid peroxidation, and antioxidant reserve capacity was determined by measuring malondialdehyde produced from myocardium incubated with the oxidant *t*-butylhydroperoxide. Hyperoxemic cardiopulmonary bypass and blood cardioplegia preserved myocardial function and produced no oxidant damage in nonhypoxemic piglets. In contrast, uncontrolled reoxygenation at oxygen tension about 400 mm Hg, followed by blood cardioplegia, resulted in marked conjugated dienes production ($42 \pm 4^*$ vs 3 ± 1) A233 nm/min/100 g during blood cardioplegic induction, reduced antioxidant reserve capacity malondialdehyde at 4 mmol/L *t*-butylhydroperoxide; $1342 \pm 59^*$ vs 958 ± 50 nmol/g protein) and caused profound myocardial dysfunction; end-systolic elastance recovered only $21\% \pm 2\%^*$ despite a blood cardioplegic regimen that was cardioprotective in nonhypoxemic piglets. Conversely, controlled cardiac reoxygenation reduced lipid peroxidation (conjugated dienes production was $2 \pm 1^{**}$), restored antioxidant reserve capacity (malondialdehyde at 4 mmol/L *t*-butylhydroperoxide; $982 \pm 88^{**}$), and allowed near-complete ($83 \pm 8\%^{**}$) functional recovery. We conclude that reoxygenation of the hypoxemic immature heart by initiating conventional hyperoxemic cardiopulmonary bypass causes oxidant damage characterized by lipid peroxidation, reduced antioxidant reserve capacity, and results in functional depression that nullifies the cardioprotective effects of blood cardioplegia. These changes can be reduced by starting cardiopulmonary bypass at the ambient oxygen tension of the hypoxemic subject and delaying subsequent reoxygenation until blood cardioplegic induction by controlled cardiac reoxygenation ($*p < 0.05$ vs control; $**p < 0.05$ vs uncontrol reoxygenation) and analysis of variance. (J THORAC CARDIOVASC SURG 1995;110:1265-73)

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With increasing frequency, intraoperative cardiac repair of cyanotic heart defects is performed with cardiopulmonary bypass (CPB) in early infancy.¹ Postoperative low output syndrome in younger patients is more frequent than in adult patients and contributes to morbidity and mortality,² despite successful anatomic correction of congenital defects. Clinically, CPB in hypoxemic infants is initiated usually at high oxygen tension (P_{O_2}) (about 400 mm Hg) without consideration of the possible cytotoxic effects of reinstitution of molecular oxygen. We have speculated that reoxygenation of cyanotic myocardium at the onset of routine CPB causes unintended surgical reoxygenation injury, which may add to subsequent intraoperative stress and compound myocardial dysfunction after CPB.³

Our hypothesis is based on reports that (1) cyanosis reduces endogenous myocardial antioxidants (e.g., glutathione peroxidase⁴) and (2) CPB per se⁵ and reoxygenation initiate potential oxidant damage via free radical generation.⁶ The clinical relevance of findings is found in reports showing myocardial lipid peroxidation in preischemic myocardial biopsy samples from cyanotic children⁷ and myocardial dysfunction in infants placed on extracorporeal membrane oxygenation.⁸

Reactive oxygen intermediates may play an integral role in the pathogenesis of reperfusion and reoxygenation injuries.⁹ We have shown also that reoxygenation damage may occur through the L-arginine-nitric oxide ($\cdot NO$) pathway.³ Production of superoxide anion ($O_2^{\cdot -}$)¹⁰ and subsequent tissue injury by reactive oxygen intermediates (hydroxyl ion, $\cdot OH$ and hydrogen peroxide, H_2O_2) are dependent on oxygen tension. Consequently, P_{O_2} at the onset of CPB may influence the occurrence of reoxygenation injury, and we have documented the potential damaging effects of abrupt hyperoxemic reoxygenation without superimposed surgical ischemia.¹¹ Furthermore, our previous studies of global and regional ischemia¹²⁻¹⁴ showed that modification of the conditions and composition of the reperfusion process limits or avoids reperfusion injury.

This study tests whether these strategies can be applied to reoxygenation of hyperoxemic infants by delaying the reoxygenation process until blood cardioplegic arrest, where the composition and condition of reoxygenation can be controlled. We evaluate the hypothesis that uncontrolled reoxygenation of hypoxemic immature hearts when starting CPB with conventional hyperoxemic P_{O_2} produces a reoxygenation injury that (1) nullifies the cardiopro-

TECTIVE effects of blood cardioplegia and (2) is avoidable by starting CPB at the ambient P_{O_2} of the hypoxemic subject and delaying reoxygenation until blood cardioplegic induction. We term this process *controlled cardiac reoxygenation*.

Material and methods

Experimental model. Twenty-one immature, 2- to 3-week-old Duroc-Yorkshire piglets (3 to 5 kg) were premedicated with 0.5 mg/kg diazepam intramuscularly, anesthetized with 30 mg/kg pentobarbital intraperitoneally followed by 5 mg/kg intravenously each hour, and the lungs ventilated on a volume-limited respirator (Servo 900D, Siemens-Elema, Solna, Sweden) via a 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, published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

An eight-electrode-equipped conductance catheter (Webster Laboratory, Baldwin Park, Calif.) was inserted through the left ventricular (LV) apex connected to a Sigma-5-DF Signal Conditioner Processor (Leycom Oegstgeest, The Netherlands). Myocardial function was evaluated by inscribing pressure-volume loops as described previously.¹⁵

A 16-gauge Teflon needle was placed in the proximal aorta to deliver cardioplegia. A heating pad was kept placed to keep the rectal temperature at 38°C. The extracorporeal circuit was primed with packed red cells from donor pigs, with calcium added to counteract citrate, hetastarch (Hespan, Dupont, Wilmington, Del.), and electrolyte solution (Plasmalyte, Baxter, Deerfield, Ill.). Hematocrit was kept between 25% to 30%, and a membrane oxygenator (16310 Membrane Oxygenator, Sarns, Ann Arbor, Mich.) was used in all studies. During CPB, mean aortic pressure was kept at about 50 mm Hg by adjusting systemic flow 100 to 120 ml/min/kg, and systemic temperature was kept at 37°C. The left ventricle was decompressed by inserting a vent through an apical stab wound.

Measurements

LV contractility. Left ventricular pressure and conductance catheter signals were amplified and digitalized to inscribe LV pressure-volume loops. A series of pressure-volume loops under variable living conditions was generated by transient occlusion of the inferior vena cava during 7 seconds of apnea under control conditions and 30 minutes after the discontinuation of CPB.¹⁶ The end-systolic pressure-volume relationship was analyzed by user-interactive videographics program (Spectrum¹⁷) on a 386 MHz IBM-PC, and LV performance was described as a slope of end-systolic elastance (E_{es}) as described previously. Postbypass LV contractility was assessed by percent recovery of prebypass control values (percent E_{es}).

Myocardial $\cdot NO$ production. $\cdot NO$ was determined in plasma by reconvertng its oxidation end products, nitrite (NO_2^-) and nitrate (NO_3^-), to $\cdot NO$ and measuring chemiluminescence after $\cdot NO$ reacts with ozone using the anitrogen oxide analyzer (Model 2108, DASIBI Environmental Corp., Glendale, Calif.).¹⁸ Blood samples were

Fig. 1. Experimental model (see text for description). BCD, Blood cardioplegia; ReO_2 , reoxygenation.

taken from coronary perfusate (blood cardioplegia) and coronary effluent (coronary sinus blood) to assess the arteriovenous difference in $\cdot NO$ during induction of blood cardioplegia. Production of $\cdot NO$ was measured by the formula: $\cdot NO$ production = $CBF \times (V - A)/100$ gm heart weight, where A and V are the respective concentrations of $\cdot NO$ measured in the aortic and coronary sinus blood during cardioplegic delivery when the cardioplegic solution was administered at a fixed coronary blood flow rate by calibrated roller pump as described under control studies.

Myocardial oxidant injury

Conjugated diene production. During blood cardioplegic induction, blood samples were taken from the aorta (blood cardioplegia and coronary effluent), coronary sinus blood, and hydroxy conjugated dienes were determined by the method of Lefnesky and coworkers¹⁹ as described previously. Myocardial conjugated diene production was calculated by the following formula and expressed as A233 nm/min/kg:

$$(CS - A) \times CBF \text{ (ml/min)/heart weight (100 gm)}$$

where CS is coronary sinus plasma conjugated diene level, A is aortic blood cardioplegic perfusate, and CBF is coronary blood flow controlled by the flow rate of blood cardioplegic delivery (milliliters per minute).

Antioxidant reserve capacity. In vitro lipid peroxidation was measured by the method of Godin and coworkers²⁰ to assess the antioxidant state or vulnerability to subsequent oxidant stress as described previously.¹⁵

Experimental groups

Twenty-one piglets underwent 60 minutes of CPB and received blood cardioplegia during 30 minutes of aortic clamping. The aorta was clamped 5 minutes after CPB

Table. Blood cardioplegia formulation

Principle	Constituent	Final concentration
Deliver oxygen	Blood	Hematocrit 20%-24%
Maintain arrest	KCl	18-20 mEq/L (induction) 10-12 mEq/L (reperfusion)
Buffer acidosis	Tromethamine	pH 7.6-7.7
Avoid edema	Glucose	Osmolarity 380-400 mOsm
Restore substrate	Glutamate	13 mmol/L
	Aspartate	13 mmol/L
	Glucose	>400 mg/dl
Limit calcium influx	Citrate phosphate dextrose	(Ca++) 0.2-0.3 mmol/L

was started, and all piglets underwent 25 additional minutes on CPB after aortic unclamping. The method of management is shown in Fig. 1 for the control and experimental groups. Previous studies document the stability of the model of the instrumented, ventilated piglet observed for 5 hours without CPB, hypoxemia, or reoxygenation.^{11, 21}

Blood cardioplegic protocol. All piglets received our standard clinical blood cardioplegic solution (hypocalcemic, alkalotic, hyperosmolar, glutamate/aspartate-enriched blood cardioplegia as shown in the Table. The blood cardioplegic protocol included (1) warm induction (KCl 20 mEq/L, 37° C, 20 ml/min/kg for 3 minutes), (2) cold replenishment (4° C, 10 ml/kg for 2 minutes), and (3) warm reperfusion (KCl 10 mEq/L, 37° C, 15 ml/min for 3 minutes) for 3 minutes (just before aortic unclamping).

Fig. 2. $\cdot$ NO production during blood cardioplegia induction in nonhypoxemic hearts (control) and during uncontrolled and controlled cardiac reoxygenation.

Control studies

Six nonhypoxemic piglets underwent 1 hour of CPB at PO_2 of about 400 mm Hg including 30 minutes of blood cardioplegia at PO_2 of about 400 mm Hg to confirm the safety of the blood cardioplegic management method (control).

Experimental studies

Fifteen piglets were made hypoxemic by lowering fraction of inspired oxygen (FiO_2) (6% to 7%) to result in an arterial oxygen tension (PaO_2) of about 25 mm Hg for up to 120 minutes before CPB was started. CPB was begun before 120 minutes of hypoxemia if mean arterial pressure fell below 30 mm Hg or arterial pH could not be kept above 7.35 by bolus intravenous infusions of $NaHCO_3$.

UNCONTROLLED REOXYGENATION. Six piglets underwent uncontrolled reoxygenation by starting CPB at the conventional hyperoxemic PO_2 of about 400 mm Hg (uncontrolled reoxygenation).

CONTROLLED CARDIAC REOXYGENATION. Nine piglets underwent controlled cardiac reoxygenation by starting CPB at the ambient PO_2 of about 25 mm Hg occurring during hypoxemia. This was done by precirculating the extracorporeal circuit at PO_2 about 25 mm Hg by blending N_2 and O_2 . Five minutes after the start of CPB, controlled cardiac reoxygenation was accomplished by raising the PO_2 of the CPB circuit to about 100 mm Hg immediately after clamping the aorta and by administering normoxemic (PO_2 about 100 mm Hg) blood cardioplegia. Arterial PO_2 was raised to about 400 mm Hg immediately after aortic unclamping.

Systemic pressure was kept at about 50 mm Hg throughout CPB, and piglets were observed for 30 minutes after CPB was discontinued; arterial blood gas and electrolytes were restored to normal before final functional measurements were made.

The heart was arrested again with a cold (4° C) blood cardioplegic solution (KCl, 30 mEq/L) to stop metabolism after final functional measurements. A LV subendocardial myocardial specimen (about 200 mg) was obtained, frozen quickly in liquid nitrogen, and stored for subsequent biochemical analyses.

Statistics. Data were analyzed with the use of the StatView V2.0 program (Abacus Concepts Inc., Berkeley, Calif.) on a Macintosh IIfx computer (Apple, Inc., Cupertino, Calif.). Analysis of variance was used for intergroup comparison. Differences were considered significant at the probability level of $p < 0.05$. Group data were expressed as mean plus or minus standard error of the mean.

Results

During hypoxemia, there was vasodilatation (the systemic vascular resistance index decreased by $32\% \pm 15\%$) and the pulmonary vasoconstriction (pulmonary vascular resistance index increased $120\% \pm 21\%$ of hypoxemic levels). The mean duration of hypoxemia averaged 73 ± 15 minutes, and premature institution of CPB was needed in 22 of 30 piglets because of hemodynamic deterioration; a similar proportion of each group required circulatory support before 120 minutes of hypoxemia had elapsed.

Myocardial $\cdot$ NO production. Hyperoxemic reoxygenation ($PO_2 > 400$ mm Hg) on CPB was associated with an 11-fold increase in $\cdot$ NO production during cardioplegic induction compared with control piglets given the same hyperoxemic blood cardioplegic solution (Fig. 2). In contrast, this marked

Conjugated Diene Production

Fig. 3. Myocardial conjugated diene production during blood cardioplegic induction in nonhypoxemic hearts (control) and during uncontrolled and controlled cardiac reoxygenation.

•NO production was reduced 94% by controlling cardiac reoxygenation by starting CPB at ambient hypoxemic PO_2 (about 25 mm Hg) and delaying reoxygenation until normoxemic blood cardioplegia was given (PO_2 about 100 mm Hg) (Fig. 2).

Myocardial conjugated diene production. Nonhypoxemic control piglets (control group) showed negligible myocardial production of conjugated dienes during warm blood cardioplegic induction. Uncontrolled reoxygenation on CPB caused a 10-fold increase in conjugated diene production (Fig. 3). Conversely, controlled cardiac reoxygenation limited conjugated diene production to levels seen in nonhypoxemic studies.

Antioxidant reserve capacity. Uncontrolled reoxygenation on CPB reduced antioxidant reserve capacity as malondialdehyde was increased significantly in myocardial homogenates incubated with the oxidant *t*-butylhydroperoxide. Conversely, piglets managed by controlled cardiac reoxygenation retained antioxidant reserve capacity at levels observed in nonhypoxemic studies (Fig. 4).

LV performance. Complete functional recovery followed 30 minutes of blood cardioplegic arrest in nonhypoxemic piglets (control) (Fig. 5). Conversely, uncontrolled reoxygenation on CPB at PO_2 about 400 mm Hg for 5 minutes before the start of the

same blood cardioplegic protocol was associated with profound myocardial dysfunction, because Ees recovered only $21\% \pm 2\%$. Controlled cardiac reoxygenation by starting CPB at the ambient (hypoxemic) PO_2 and delivering a normoxic blood cardioplegic solution resulted in near-complete functional recovery, because Ees returned to $83\% \pm 3\%$ of control values.

Discussion

This study documents the role of reoxygenation injury in the pathogenesis on postbypass cardiac dysfunction and addresses the importance of controlling PO_2 at the onset of CPB as a cardioprotective strategy for cyanotic infants. It confirms that (1) reoxygenation of hypoxemic immature hearts at the onset of CPB causes oxygen-mediated damage characterized by lipid peroxidation, impairment of antioxidant reserve capacity, and functional depression, (2) this unintended injury nullifies the cardioprotective effects of a blood cardioplegic protocol that is safe and nonhypoxemic piglets, and (3) the deleterious effects of reoxygenation can be avoided by starting CPB at the ambient PO_2 of the hypoxemic subject and delaying reoxygenation until blood cardioplegic induction; *controlled cardiac reoxygenation*.

Glutamate- and aspartate-enriched hypocalcemia,

Fig. 4. Antioxidant reserve capacity in cardiac homogenates after blood cardioplegia in nonhypoxemic hearts (control) and after uncontrolled and controlled cardiac reoxygenation. *MDA*, Malondialdehyde.

alkalotic, hyperosmolar blood cardioplegia used in this study is employed routinely in high-risk adult patients²² and has been studied experimentally in energy- and substrate-depleted ischemic hearts.^{23,24} The same blood cardioplegic protocol that has been shown safe in non-hypoxemic puppies was ineffective if applied after a period of reoxygenation on CPB produced by initiating hyperoxemia in hypoxemic subjects. Conversely, starting CPB at the ambient P_{O_2} and delaying reoxygenation and initiating it more gradually by delivering a normoxemic blood cardioplegic solution reduced cardiac damage substantially by avoiding lipid peroxidation, retaining antioxidant reserve capacity, and facilitating functional recovery. These findings are consistent with our previous studies showing that controlled reperfusion after global^{12,13} and regional¹⁴ ischemia limited reperfusion injury and that documented oxygen-mediated damage as an important aspect of this process.²⁵ These present data suggest that a similar salutary effect occurs in reoxygenation injury if the reintroduction of molecular O_2 is delayed until the blood cardioplegic solution is administered; we term this process *controlled cardiac reoxygenation*.

We speculate that several factors contribute to

the benefits of blood cardioplegic reoxygenation including (1) potassium cardioplegic arrest, (2) modification of the blood cardioplegic content (i.e., alkalosis and hypocalcemia), (3) supplementation with glutamate and aspartate, and (4) lowering the P_{O_2} of blood cardioplegia and have defined their individual benefits previously.^{13,26} We documented recently³ that $\cdot NO$, which is normally of physiologic benefit as endothelium-derived relaxation factor, contributes to reoxygenation injury presumably through interacting with superoxide anion ($O_2^{\cdot -}$) to form peroxynitrite ($OONO^-$) and the highly toxic hydroxyl radical ($\cdot OH$) as described by Beckman and colleagues.²⁷

Several components of the controlled reoxygenation strategy (e.g., lowering P_{O_2} ,²⁸ hypocalcemia,²⁹ alkalosis,³⁰ and amino acid supplementation³¹⁻³⁴) can also limit $\cdot NO$ production and subsequent oxidant injury so that this new pathway of oxygen-mediated damage may be rendered less damaging by the controlled reoxygenation strategy. This may be especially true since sudden reoxygenation is associated with marked $O_2^{\cdot -}$ and $\cdot NO$ production,^{10,28} which may be limited by more gradual reoxyge-

Fig. 5. Recovery of contractile function, expressed as percent of control Ees after blood cardioplegia in nonhypoxemic hearts (control), and after controlled and uncontrolled cardiac reoxygenation.

ation (normoxemia rather than hyperoxemic introduction of molecular oxygen) and the use of blood cardioplegic solution containing components that reduce $\cdot\text{NO}$ synthase activity. Fig. 2 shows that $\cdot\text{NO}$ production was limited markedly by controlled cardiac reoxygenation.

Antioxidant reserve capacity was measured to assess the vulnerability to subsequent oxidant stress, because this measurement reflects the status of endogenous defenses (e.g., superoxide dismutase, glutathione).²⁰ Severe reduction of endogenous antioxidants persisted after reoxygenation on CPB, which suggests that reoxygenated myocardium may become more susceptible to subsequent ischemic insults during cardiac repair and exhibit postbypass myocardial dysfunction. Conversely, controlled cardiac reoxygenation retained normal antioxidant reserve capacity (perhaps accounting for the better tolerance to the period of aortic clamping imposed to simulate surgical conditions).

Clinical implications. This study suggests that oxidant injury may occur if the conventional practice of starting hyperoxemic CPB in hypoxemic immature hearts is followed and emphasizes the impor-

tance of controlling the process of initial reoxygenation. Inherent weaknesses of the study include (1) failure to distinguish the effects of gradual reoxygenation without cardioplegic administration, (2) failure to separate the roles of prebypass ischemia, which is common in the ventilator hypoxemia model and requires premature institution of bypass more than 70% of studies before 2 hours, and (3) the use of an acute rather than a chronic hypoxemic model.

Our previous studies²¹ document that gradual reoxygenation (over a 1-hour period) does not limit reoxygenation damage, but the end point has always been hyperoxemia. We have shown also that reoxygenation injury occurs even if hemodynamic deterioration and acidosis of ventilator hypoxemia are avoided by using a model of hypoxemia on bypass.¹⁵ Chronic cyanosis results in myocardial metabolic adaptations to maintain normal metabolism³⁵ that do not occur in acute preparations. These adaptations notwithstanding, the results in these short-term studies are similar to those observed clinically; Teoh and coworkers⁴ showed that chronic cyanosis depletes the myocardium of endogenous antioxidants (e.g., glutathione peroxidase, superoxide dis-

mutase). Del Nido and coworkers⁷ reported evidence of lipid peroxidation of preischemic myocardial biopsy samples from cyanotic children placed on CPB, and myocardial dysfunction progressing to myocardial stun occurs in cyanotic infants placed on extracorporeal membrane oxygenation without surgical ischemia.⁸

We recognize that the profound myocardial dysfunction measured in this study seems to conflict with the seemingly uneventful postoperative condition after repair of cyanotic defects (e.g., pulmonary stenosis with ventricular septal defect). All piglets could maintain satisfactory hemodynamics without inotropic support, and we quantified contractile changes by sensitive indexes (Ees) that cannot be appreciated from the bedside. We suspect that reoxygenation injury may further increase the vulnerability to subsequent ischemic/reperfusion damage when aortic clamping is needed for surgical repair and may contribute to postoperative cardiac dysfunction. Subsequent long-term studies are needed to determine if reoxygenation injury occurs in hearts adapted to hypoxemia because the strategies used in this study are applicable readily during routine cardiac operations. Controlled cardiac reoxygenation may improve the outcome of cyanotic patients undergoing repair of congenital defects causing hypoxemia if our observations are confirmed.

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