STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY: WITH AORTIC CLAMPING

X. Exogenous antioxidants to avoid nullification of the cardioprotective effects of blood cardioplegia

This study tests the hypothesis that reoxygenation of cyanotic immature hearts when starting cardiopulmonary bypass produces an "unintended" reoxygenation injury that (1) nullifies the cardioprotective effects of blood cardioplegia and (2) is avoidable by adding antioxidants *N*-(2-mercaptopropionyl)-glycine plus catalase to the cardiopulmonary bypass prime. Twenty immature piglets (2 to 3 weeks) underwent 30 minutes of aortic clamping with a blood cardioplegic solution that was hypocalcemic, alkalotic, hyperosmolar, and enriched with glutamate and aspartate during 1 hour of cardiopulmonary bypass. Of these, six piglets did not undergo hypoxemia (blood cardioplegic control) and 14 others remained hypoxemic (oxygen tension about 25 mm Hg) for up to 2 hours by lowering ventilator fraction of inspired oxygen before reoxygenation on cardiopulmonary bypass. The primary solution of the cardiopulmonary bypass circuit was unchanged in eight piglets (no treatment) and supplemented with the antioxidants *N*-(2-mercaptopropionyl)-glycine (80 mg/kg) and catalase (5 mg/kg) in six others (*N*-(2-mercaptopropionyl)-glycine and catalase). Myocardial function (end-systolic elastance), lipid peroxidation (myocardial conjugated diene production), and antioxidant reserve capacity were evaluated. Blood cardioplegic arrest produced no biochemical or functional changes in nonhypoxemic control piglets. Reoxygenation caused an approximate 10-fold increase in conjugated production that persisted throughout cardiopulmonary bypass, lowered antioxidant reserve capacity 86% ± 12%, and produced profound myocardial dysfunction, because end-systolic elastance recovered only 21% ± 2%. Supplementation of the cardiopulmonary bypass prime with *N*-(2-mercaptopropionyl)-glycine and catalase reduced lipid peroxidation, restored antioxidant reserve capacity, and allowed near complete functional recovery (80% ± 8%). Lipid peroxidation (conjugated diene) production was lower during warm blood cardioplegic reperfusion than during induction in all reoxygenated hearts, which suggests that blood cardioplegia did not injure reoxygenated myocardium. We conclude that reoxygenation of the hypoxemic immature heart causes cardiac functional and antioxidant damage that nullifies the cardioprotective effects of blood cardioplegia that can be avoided by supplementation of the cardiopulmonary bypass prime with antioxidants (*$p < 0.05$ vs blood cardioplegic control; **$p < 0.05$ vs reoxygenation). (J THORAC CARDIOVASC SURG 1995;110:1245-54)**

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Blood cardioplegia (BCP) is reportedly less cardioprotective in cyanotic than in noncyanotic patients,[1] presumably because hypoxemic immature hearts are more susceptible to ischemic/reperfusion injury.[2,3] This vulnerability may in part account for the early ventricular dysfunction after successful anatomic correction of cyanotic congenital defects[4] and the higher morbidity and mortality than in nonhypoxemic adult patients.[5-7] Our recent studies[8,9] focus on the deleterious cardiac effects of restoring molecular oxygen by initiating cardiopul-

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monary bypass (CPB) in previously hypoxemic immature hearts. Myocardial damage occurred in the absence of ischemic/reperfusion stress (aortic clamping); this was interpreted to suggest that an unintended surgical reoxygenation injury develops in the cyanotic myocardium at the onset of routine CPB. Reoxygenation-induced myocardial depression was characterized by lipid peroxidation and reduced cardiac antioxidant reserve capacity and occurred independent of the method of causing hypoxemic/reoxygenation stress.[10] These observations in the acute hypoxemic model are consistent with clinical reports showing myocardial lipid peroxidation and reduced antioxidant enzyme activity (glutathione peroxidase) in preischemic myocardial biopsy samples from cyanotic children placed on CPB.[11, 12] The aforementioned experimental and clinical findings led us to hypothesize that this initial oxidant injury may become additive to subsequent intraoperative oxidative stress (i.e., aortic clamping) and compound post-CPB dysfunction during surgical repair of congenital defects causing cyanosis.

Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl ion (·OH) are reactive oxygen intermediates. Of these, ·OH is the most cytotoxic that causes lipid peroxidation and protein thiol oxidation,[13] which leads to myocardial and endothelial cell injury on reoxygenation.[14, 15] Hydroxyl radical may be produced through both the iron-catalyzed Haber-Weiss reaction[16] and the L-arginine–nitric oxide pathway[8] and may have other deleterious consequences.[17, 18] Our prior studies show hypoxemia/reoxygenation damage without associated aortic clamping can be limited by priming the extracorporeal circuit with the exogenous antioxidant *N*-(2-mercaptopropionyl)-glycine, which is a powerful scavenger of ·OH[14, 15] and hypochlorous acid [HOCl],[17] and catalase, which scavenges H_2O_2.[19]

This study incorporates an interval of aortic clamping to simulate the ischemic/reperfusion stress coincident with surgical repair of lesions causing cyanosis in immature hearts. Myocardial management was with a hypocalcemic, alkalotic, glutamate/aspartate-enriched BCP solution shown previously to restore mechanical function to energy-depleted hearts subjected to regional and global ischemia/reperfusion injury in the experimental[20] and clinical setting.[21] Its safety in hypoxemic immature puppy hearts is established,[22] but its cardioprotective capacity in the porcine myocardium (which closely simulates the human heart[23]) and its ability to restore metabolism and function to myocardium subjected hypoxemic/reoxygenation are unproved.

These experiments in an in vivo piglet model of hypoxemia with subsequent reoxygenation on CPB and aortic clamping with BCP were done to test the hypotheses that (1) a brief interval (5 minutes) of reoxygenation at the initiation of bypass causes sufficient myocardial injury to nullify the cardioprotective effects of BCP, and (2) these deleterious reoxygenation changes can be offset by adding *N*-(2-mercaptopropionyl)-glycine amd catalase to the priming fluid of the extracorporeal circuit.

Material and methods

Experimental model. Twenty-five immature, 2- to 3-week-old Yorkshire-Duroc 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). The surgical preparation, including vessel cannulation for CPB and placement of the catheter for monitoring and blood sampling and prime of the CPB circuit, is similar to that reported previously.[10]

Physiologic and biochemical determinations. Hemodynamic measurements were made before the start of hypoxemia (control), every 15 minutes during hypoxemia, and 15 and 30 minutes after discontinuation of CPB. Cardiac output was determined by duplicate injections of 1 ml of 4° C cold saline solution into a central venous catheter. Cardiac index (CI), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), and left ventricular stroke work index (LVSWI) were calculated with the following equations:

$$\text{CI (ml/min/kg)} = \text{CO/body weight (kg)}$$

$$\text{SVRI (mm Hg} \cdot \text{min} \cdot \text{L}^{-1} \cdot \text{kg)} = (\text{MAP} - \text{CVP})\ (\text{mm Hg}) \cdot \text{CO}^{-1}\ (\text{L/min}) \cdot \text{BW (kg)}$$

$$\text{PVRI (mm Hg} \cdot \text{min} \cdot \text{L}^{-1} \cdot \text{kg)} = (\text{PAP} - \text{LAP})\ (\text{mm Hg}) \cdot \text{CO}^{-1}\ (\text{ml/min}) \cdot \text{BW (kg)}$$

$$\text{LVSWI (g} \cdot \text{m/kg)} = (\text{MAP} - \text{LAP}) \times \text{CO (ml/min)} \times 0.0136/(\text{HR} \times \text{body weight [kg]})$$

where MAP is mean aortic pressure, PAP is mean pulmonary artery pressure, LAP is mean left atrial pressure, CVP is central venous pressure, CO is cardiac output in milliliters per minute, and HR is heart rate.

Left ventricular (LV) performance was evaluated by inscribing pressure-volume loops with an LV five-electrode conductance catheter as described previously, and CO was determined by duplicate injections of 1 ml of 4° C

Table. *Hemodynamic parameters during hypoxia and reoxygenation*

	Control	*Hypoxia (15 min)*	*Hypoxia (30 min)*	*End hypoxia*	*15 min off CPB*	*30 min off CPB*
CI (ml/min/kg)						
Reox + BCP	118 ± 6	95 ± 28	117 ± 21	109 ± 27	68 ± 9	85 ± 14
+ MPG/CAT	132 ± 19	128 ± 29	102 ± 33	129 ± 36	98 ± 30	99 ± 15
SWI (g × m/kg)						
Reox + BCP	0.49 ± 0.07	0.40 ± 0.13	0.38 ± 0.10	0.39 ± 0.08	0.14 ± 0.04	0.21 ± 0.04
+ MPG/CAT	0.51 ± 0.11	0.37 ± 0.12	0.40 ± 0.14	0.34 ± 0.15	0.35 ± 0.05*	0.36 ± 0.06*
SVRI (mm Hg · min · L^{-1} · kg)						
Reox + BCP	537 ± 44	441 ± 73	388 ± 70	348 ± 62	544 ± 95	571 ± 139
+ MPG/CAT	434 ± 47	342 ± 50	333 ± 48	332 ± 24	367 ± 71	387 ± 45
PVRI (mm Hg · min · L^{-1} · kg)						
Reox + BCP	72 ± 8	264 ± 34	203 ± 24	180 ± 11	289 ± 64	312 ± 53
+ MPG/CAT	70 ± 13	230 ± 45	177 ± 35	177 ± 45	131 ± 40	124 ± 36*

Reox + BCP, All piglets were reoxygenated on CPB and received BCP; *+MPG/CAT,* those piglets reoxygenated on CPB, receiving BCP, where the priming fluid of the CPB circuit was supplemented with mercaptropropionylglycine (80 mg/kg) and catalase (50,000 units/kg).

*$p < 0.05$ versus reoxygenation + BCP. (Reox + BCP)

saline solution into the right atrium through a thermodilution probe placed into the main pulmonary artery and connected to a cardiac computer (Model 9520A, American Edwards Laboratory, Santa Ana, Calif.). Results were expressed as stroke work index (SWI) in g × m/kg to normalize for body weight.

Myocardial oxidant injury

Plasma conjugated dienes. Hydroxy conjugated diene (CD) concentration in coronary artery and coronary sinus blood was determined as described by Lefnesky and coworkers.[24]

CD production. Aliquots of coronary artery perfusate (BCP) and coronary venous effluent were sampled to determine CD production. CD production was calculated by the following formula; CBF (V − A)/100 gm heart weight, where A and V are the concentration of CDs in the BCP perfusate and coronary sinus effluent respectively, and coronary blood flow (CBF) is the BCP flow rate in milliliters per minute.

Antioxidant reserve capacity. We assessed the myocardial antioxidant state in vitro by incubating myocardial homogenates with 0 to 4 mmol of the oxidant *t*-butylhydroperoxide for 30 minutes as described previously by Godin and colleagues.[25]

Protocol for BCP myocardial management. The BCP formulation was developed experimentally[26, 27] and is used clinically.[28, 29] The BCP protocol included induction with warm cardioplegia (37° C) for 3 minutes followed by cold cardioplegia for 2 minutes (4° C), and a 3-minute reperfusion with 37° C BCP just before aortic unclamping. Infusion rate was regulated by calibrated roller pump at 10 ml/min/kg initially to produce arrest and 5 ml/min/kg thereafter with aortic pressure averaging about 50 mm Hg.

Experimental groups

Nonhypoxemic studies. Five normoxemic piglets were anesthetized, instrumented, and observed over 5 hours without CPB to provide control hemodynamic and biochemical data. Six other piglets underwent CPB at oxygen tension (Po_2) about 400 mm Hg and BCP arrest without preceding hypoxemia.

Hypoxemic/reoxygenation studies. Fourteen piglets were made hypoxemic by lowering inspired oxygen fraction (Fio_2) to 6% to 7% to reduce Po_2 to about 25 mm Hg for up to 120 minutes, followed by reoxygenation on CPB at Po_2 about 400 mm Hg, and 30 minutes of BCP arrest. The aorta was clamped 5 minutes after CPB was started to simulate what is done clinically. Reoxygenation before 120 minutes was begun if either mean arterial pressure fell below 30 mm Hg and was unresponsive to volume infusion or arterial pH could not be kept above 7.3 by three bolus intravenous infusions of $NaHCO_3$.

Hypoxemic/Reoxygenation without treatment studies (Reox + BCP). Eight piglets underwent the aforementioned protocol without changing the priming fluid in the extracorporeal circuit.

N-(2-mercaptopropionyl)-glycine and catalase. In six piglets the priming fluid of the CPB circuit was supplemented with *N*-(2-mercaptopropionyl)-glycine (80 mg/kg) plus catalase (50,000 units/kg/L).

Statistics. Data were analyzed with the use of the StatView V2.0 program (Abacus Concepts Inc., Berkeley, Calif.) on a Macintosh IIci computer (Apple, Inc., Cupertino, Calif.). Analysis of variance was used for intergroup comparison, and the paired Student *t* test was used for comparison of variables within experimental groups. The relationship between functional impairment (percent Ees) and lipid peroxidation (CDs) was tested by linear regression analysis. 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

Hemodynamic results. All hemodynamic variables remained stable during 5 hours of observation in instrumented piglets that did not undergo CPB, and pre- and post-CPB measurements were comparable after 60 minutes of CPB, including 30 minutes of aortic clamping with BCP. The Table shows the

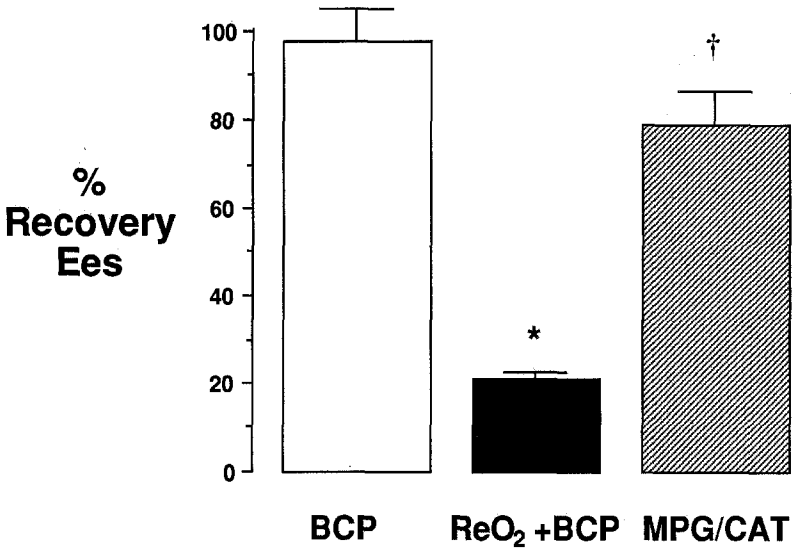

Fig. 1. Recovery of LV contractility, expressed as percent of control end-systolic elastance *(Ees)* in nonhypoxemic piglets receiving BCP, after reoxygenation *(ReO_2)* followed by BCP, and when MPG and CAT were added to the extracorporeal circuit prime before CPB was started (see text for description). *$p <$ 0.05 vs BCP and MPG/CAT; †$p < 0.05$ vs ReO_2 + BCP.

hemodynamic changes during control, hypoxemia, and after CPB was discontinued in piglets undergoing hypoxemia and reoxygenation on CPB and BCP management. The response to hypoxemia was systemic vasodilatation and pulmonary vasoconstriction, and the mean duration of hypoxemia was 76 minutes ± 11 minutes and 72 minutes ± 13 minutes, respectively, in piglets reoxygenated on bypass with the unmodified priming fluid (no treatment) and when the CPB prime was supplemented with MPG/CAT. Premature start of CPB because of hemodynamic deterioration was needed in 10 of 14 piglets.

LVSWI was calculated after transfusion to LAP 8 mm Hg before and after CPB, and recovered completely in nonhypoxemic piglets. Conversely, LVSWI recovered only 42% in untreated piglets but returned to 70% of CPB values when *N*-(2-mercaptopropionyl)-glycine and catalase was added to the CPB prime ($p < 0.05$). However, CIs were similar in both groups, but SVRI was slightly lower after *N*-(2-mercaptopropionyl)-glycine and catalase treatment.

LV performance. Complete functional recovery occurred in nonhypoxemic piglets managed by BCP during 30 minutes of aortic clamping. Conversely, end-systolic elastance (Ees) recovered only 21% ± 2% ($p < 0.05$ vs BCP, Fig. 1) after reoxygenation on CPB before application of the same BCP regimen. Post-CPB recovery of Ees was 80% ± 8% with this myocardial management method during aortic clamping when CPB was initiated with a prime containing *N*-(2-mercaptopropionyl)-glycine and catalase ($p < 0.05$ vs reoxygenation plus CPB).

PVRI. PVRI remained unchanged during the 5-hour observation period but increased 156% in nonhypoxemic piglets subjected to 60 minutes of CPB (213 ± 30 vs 83 ± 12 mm Hg · min · L^{-1} · kg, $p <$ 0.05 vs control) and rose 345% in piglets reoxygenated on CPB (312 ± 53 mm Hg · min · mL^{-1} · kg, $p < 0.05$ vs reoxygenation and BCP). The lowest post-CPB PVRI occurred when *N*-(2-mercaptopropionyl)-glycine and catalase were added to the CPB prime, with values returning to near control levels (Fig. 2).

Oxidant damage. Coronary sinus conjugated dienes remained normal, and no CD production occurred in nonhypoxemic piglets undergoing CPB with 30 minutes of BCP management. In contrast, CD production increased 10-fold during cardioplegic induction and persisted to a lesser extent during reperfusion after reoxygenation on CPB (Fig. 3) and returned to normal toward the end of CPB. The addition of *N*-(2-mercaptopropionyl)-glycine and catalase to the CPB prime reduced CD production during both induction and reperfusion (Fig. 3) and coronary sinus plasma CD returned to control levels after aortic unclamping.

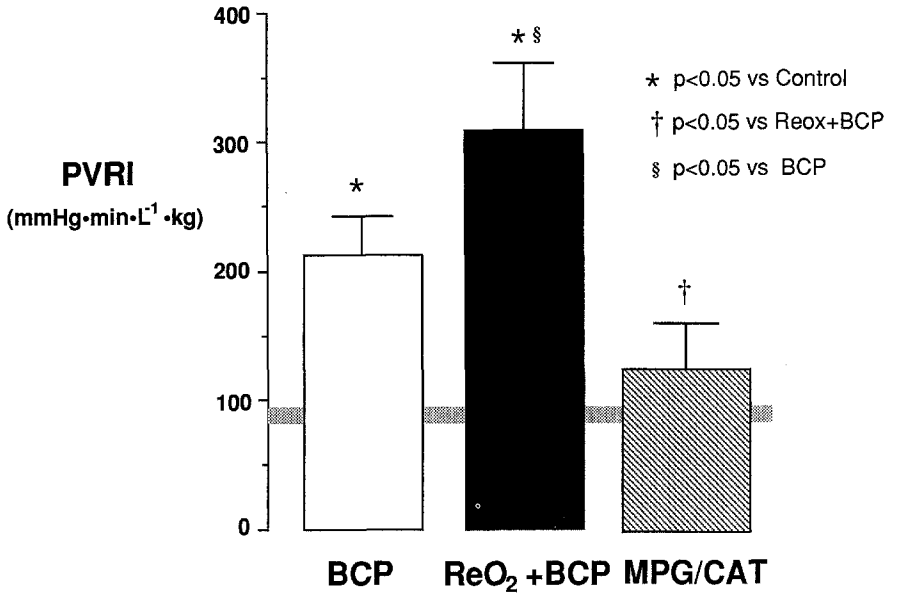

Fig. 2. PVRI after CPB was discontinued. *ReO_2*, Reoxygenation (see text for description). *Shaded bar* indicates control values for PVR in instrumented piglets that did not undergo CPB.

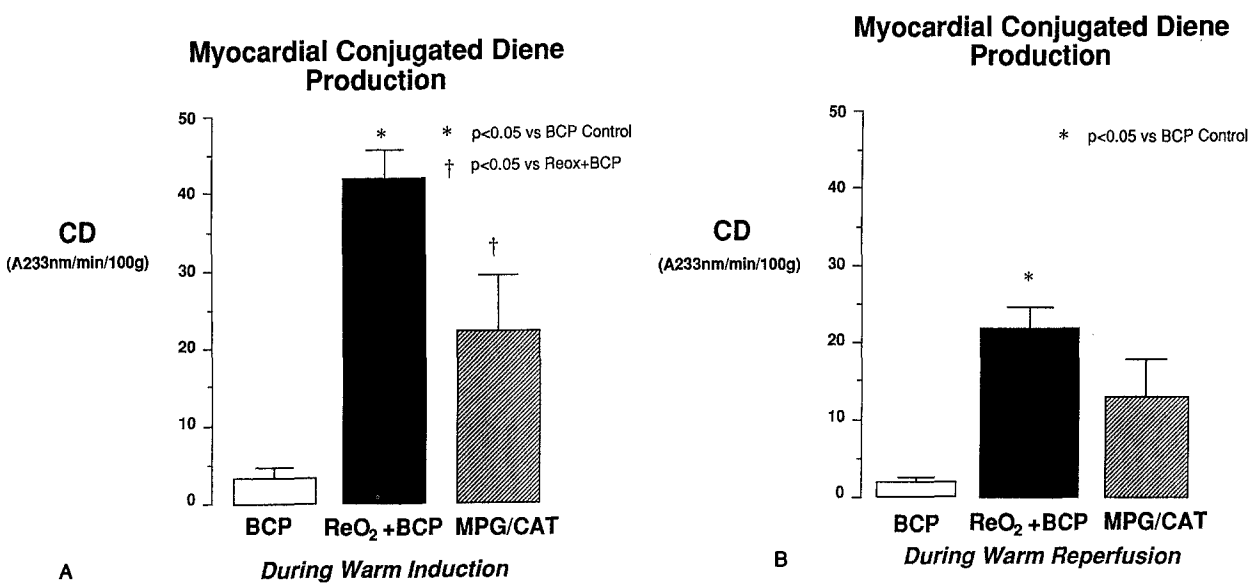

Fig. 3. A, Myocardial CD production during BCP induction and reperfusion **B.** *ReO_2*, Reoxygenation (see text for description).

Biochemistry results

Antioxidant reserve capacity. Antioxidant reserve capacity remained at control levels in nonhypoxemic piglets undergoing CPB and 30 minutes of aortic clamping with BCP, whereas 86% ± 14% more malendialdehyde was produced than control values after incubation with 4 mmol *t*-butylhydroperoxide after reoxygenation with the same myocardial management protocol in untreated piglets (Fig. 4). In contrast, post-CPB values for antioxidant reserve

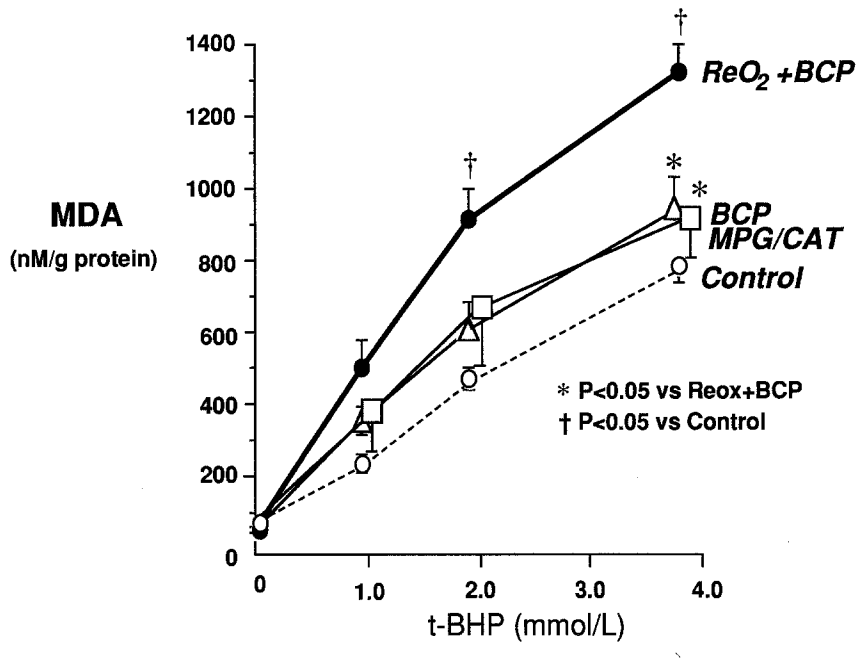

Fig. 4. Antioxidant reserve capacity measured after 30-minutes post-CPB observation period. *ReO_2*, Reoxygenation.

capacity after reoxygenation with an extracorporeal circuit primed with *N*-(2-mercaptopropionyl)-glycine and catalase were comparable with those in nonhypoxemic piglets undergoing the same BCP protocol.

Discussion

These data provide further confirmation of the role of reoxygenation injury in previously hypoxemic hearts in the pathogenesis of postbypass dysfunction[8] in the setting of an otherwise safe method of myocardial management during aortic clamping.[22] A brief (i.e., 5 minutes) interval of reoxygenation on CPB caused sufficient oxidant damage to nullify the cardioprotective effects of an otherwise safe BCP strategy.[30] These deleterious consequences of reoxygenation could be offset substantially by adding antioxidants to the priming fluid of the extracorporeal circuit to counteract the oxidant stress of abrupt reoxygenation.

Background. Chronic cyanosis depletes the human myocardium of endogenous antioxidant enzyme activity (i.e., glutathione peroxidase and superoxide dismutase [SOD])[12] despite several metabolic adaptations,[31] which maintain aerobic metabolism in cyanotic myocardium. These biochemical consequences of hypoxemia can be reproduced experimentally in the acute setting[10] and may contribute to the vulnerability of chronic cyanotic hearts to intraoperative aortic clamping as shown both experimentally and clinically.[1-3] The aforementioned observations led us to speculate that reduced endogenous antioxidant defenses may reduce the threshold for triggering oxidative injury when a burst of oxygen free radicals occurs when CPB is initiated in previously cyanotic hearts and thereby potentially limit the cardioprotective and resuscitative effects of efficient myocardial management techniques during aortic clamping.[27] The adverse mechanical consequences of reoxygenation are perhaps most evident from reports of myocardial dysfunction progressing to myocardial stunning (no aortic pulsations) after cyanotic infants are placed on extracorporeal oxygenation for pulmonary disorders, because they occur without superimposed surgical ischemia.[32] These observations lead us to suspect an even greater oxidant challenge to occur in cyanotic patients who require surgical ischemia during intraoperative repair and may therefore contribute to their higher morbidity and mortality compared with nonhypoxemic patients undergoing similar myocardial management techniques.[5-7]

The myocardial management strategy of glutamate/aspartate-enriched BCP is an experimentally

sound and clinically relevant method of preventing ischemic damage in adult and pediatric patients that also resuscitates energy and substrate-depleted hearts subjected to ischemic/reperfusion injury.[22, 26, 29, 33] Its ineffectiveness to reverse hypoxemic/reoxygenation damage was an unexpected finding and may be the result of the more severe oxidant stress imposed by reoxygenation versus reperfusion, as shown recently.[34] The pathophysiology of reoxygenation and reperfusion damage differs in that hypoxemia washes away reducing equivalents (lactate) that may counteract oxidant damage during reperfusion[35] so that the consequences of superimposed ischemia/reperfusion stress become amplified when the aorta is clamped immediately after reoxygenation on CPB. In this study CD production was lower during warm reperfusion than during warm induction, and the post-CPB reduction of antioxidant reserve capacity was similar to that observed in our previous study of reoxygenation on CPB without superimposed ischemia.[10] These observations suggest that the cardioprotective effect of the myocardial management strategy to "prevent" ischemic damage was retained but that reoxygenation injury limited its capacity to reverse the damage produced by reintroduction of molecular oxygen.

The most pronounced evidence of lipid peroxidation (CD production) occurred during warm BCP induction 5 minutes after reoxygenation on CPB. We suspect the delayed sampling (until CBF was controlled during cardioplegic administration) led to a systemic underestimation of oxidant damage, which likely followed the burst of free radical activity immediately on reintroduction of molecular oxygen[36-38] when CPB was started. Studies of oxidant damage during ischemia/reperfusion show a rapid buildup of products of lipid peroxidation on reoxygenation, with subsequent tissue depletion,[39] and yet plasma CDs in coronary sinus blood did not increase until 35 minutes of reoxygenation on bypass. We suspect that CBF autoregulated from the initial hyperemic phase, thereby accounting for a higher plasma concentration despite evidence of reduced production during myocardial management with BCP. Alternatively, the cessation of the cardioplegic infusion provided by BCP protection may have allowed a resurgence of oxidant damage when unmodified blood was restored when the aorta was unclamped. Unfortunately, serial myocardial biopsy samples were not obtained and CBF was not measured either immediately before or after aortic clamping and unclamping to allow determination of which explanation was correct.

The antioxidants *N*-(2-mercaptopropionyl)-glycine and catalase were selected for study because of our prior experience with their effectiveness in limiting reoxygenation damage in the absence of superimposed surgical ischemia.[8] *N*-(2-mercaptopropionyl)-glycine is an ·OH scavenger that also counteracts hypochloric acid produced by activated neutrophils and impedes the cytotoxic effects of reactive oxygen intermediates that cause lipid peroxidation and protein thiol oxidation.[17] This substance also may interfere with oxidant damage via the L-arginine–·NO pathway because of its action on peroxynitrite ($OONO^-$).[40] Catalase scavenges H_2O_2 radicals, which have deleterious effects other than via iron-catalyzed hydroxyl radical formation, including production of thromboxine A_2[18] and myoglobin-H_2O_2–induced oxidants.[17] The combined effects of *N*-(2-mercaptopropionyl)-glycine and catalase in reducing lipid peroxidation, retarding depletion of antioxidant reserve capacity, and improving functional recovery after reoxygenation are consistent with similar benefits in the ischemic/reperfusion model.[14, 15]

The results imply that assurance of satisfactory blood concentration of antioxidants at the initiation of reoxygenation counteracted the oxidant damage that otherwise would have occurred and allowed the cardioprotective effects of the myocardial management technique to remain operative. Clearly, alternative antioxidant approaches (deferoxamine, CoQ_{10}, SOD, allopurinol, etc.) may also limit reoxygenation damage, but the experimental design was intended to establish the role of antioxidants in the CPB prime rather than to compare different compounds with alternate mechanisms of action.

Reoxygenation damage is a biologic phenomena that is not confined to the myocardium. The current findings of increased PVRI in nonhypoxemic piglets after CPB were shown previously[41] and may reflect both the recognized adverse consequences of CPB in activating complement,[6, 42] neutrophils,[43] the arachidonic acid pathway,[44] and ischemic/reperfusion damage because pulmonary blood flow is diverted from the lung during cardiopulmonary bypass and restored when CPB is discontinued.

Reoxygenation caused a further augmentation of PVRI (Fig. 2), which suggests that reoxygenation-induced endothelial damage may have occurred. Both H_2O_2 and subsequent ·OH production may be principal effectors of endothelial dysfunction and subsequent morphologic disruption,[45] because recent studies[46] show that a brief exposure to H_2O_2

causes dose-dependent impairment of in vitro pulmonary artery endothelial cell function. Our findings that PVRI returned to near normal levels after *N*-(2-mercaptopropionyl)-glycine and catalase may indicate that the noncardiac effects of ischemia/reperfusion and hypoxemia/reoxygenation were counteracted by these antioxidants. Transient post-CPB pulmonary endothelial dysfunction raises PVRI by impairing release of endogenous endothelial-derived relaxation factor, thereby explaining why inhaled nitric oxide can reduce PVRI in post-cyanotic patients undergoing surgical correction of congenital defects causing cyanosis.[47] The control data in nonhypoxemic piglets provide further evidence of the cardioprotective effects of hypocalcemic solutions in the immature heart.[48, 49] The beneficial effects of selected antioxidants *N*-(2-mercaptopropionyl)-glycine and catalase on reoxygenation damage are consonant with their usefulness in the ischemic/reperfusion model[14, 15] in that *N*-(2-mercaptopropionyl)-glycine and catalase-treated piglets showed less evidence of lipid peroxidation and expenditure of antioxidant reserve capacity and better functional recovery than occurred in untreated piglets undergoing a comparable myocardial management protocol.

The questionable relevance of the acute hypoxemic model to clinical chronic cyanosis is acknowledged, but our findings are consistent with reports in chronic cyanotic animals[3] and in hypoxemic patients undergoing surgical repair.[1, 11, 12, 50] Our quantification of myocardial dysfunction by inscribing pressure-volume loops appears inconsistent with clinical observations of "uneventful recovery" in cyanotic patients (i.e., pulmonary stenosis or atresia and ventricular septal defect) who undergo surgical repair after a brief period of CPB. Temporary mild inotropic support is, however, somewhat routine after surgery despite the brief period of bypass. Extracorporeal circulation could be discontinued in all piglets without inotropic support, and myocardial depression was quantified by the more comprehensive method of inscribing pressure-volume loops that is not applied clinically. Furthermore, we did not administer inotropic drugs to determine if normal function could be restored to match the routine perioperative management. We conclude that reoxygenation injury may add an otherwise avoidable incremental adverse factor to the surgical correction of congenital defects causing cyanosis, because both the duration of CPB and surgical ischemia may be prolonged during repair of congenital defects. However, extrapolation of these findings to clinical events will require study in chronic animals, as well as quantification of the markers of lipid peroxidation and reduced antioxidant reserve capacity in cyanotic patients placed on CPB.

The findings of these short-term experiments showing that reoxygenation injury can nullify the cardioprotective effects of an otherwise safe myocardial management technique and that adverse cardiac and pulmonary effects of reoxygenation can be counteracted by supplementation of the extracorporeal prime with antioxidants will hopefully lead to increased appreciation that the initiation of CPB in hypoxemic infants may not be an innocuous event. It can produce adverse changes that could be modified by better understanding of the pathophysiology of reoxygenation injury.

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