STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY: WITH AORTIC CLAMPING

XIII. Interaction between oxygen tension and cardioplegic composition in limiting nitric oxide production and oxidant damage

This study tests the interaction between oxygen tension and cardioplegic composition on nitric oxide production and oxidant damage during reoxygenation of previously cyanotic hearts. Of 35 Duroc-Yorkshire piglets (2 to 3 weeks, 3 to 5 kg), six underwent 30 minutes of blood cardioplegic arrest with hyperoxemic (oxygen tension about 400 mm Hg), hypocalcemic, alkalotic, glutamate/aspartate blood cardioplegic solution during 1 hour of cardiopulmonary bypass without hypoxemia (control). Twenty-nine others were subjected to up to 120 minutes of ventilator hypoxemia (oxygen tension about 25 mm Hg) before reoxygenation on CPB. To simulate routine clinical management, nine piglets underwent *uncontrolled cardiac reoxygenation*, whereby cardiopulmonary bypass was started at oxygen tension of about 400 mm Hg followed by the aforementioned blood cardioplegic protocol 5 minutes later. All 20 other piglets underwent *controlled cardiac reoxygenation*, whereby cardiopulmonary bypass was started at the ambient oxygen tension (about 25 mm Hg), and reoxygenation was delayed until blood cardioplegia was given. The blood cardioplegia solution was kept normoxemic (oxygen tension about 100 mm Hg) in 10 piglets and made hyperoxemic (oxygen tension about 400 mm Hg) in 10 others. The cardioplegic composition was also varied so that the cardioplegic solution in each subgroup contained either KCl only (30 mEq/L) or components that theoretically inhibit nitric oxide synthase by including hypocalcemia, alkalosis, and glutamate/aspartate. Function (end-systolic elastance) and myocardial nitric oxide production, conjugated diene production, and antioxidant reserve capacity were measured. Blood cardioplegic arrest without hypoxemia did not cause myocardial nitric oxide or conjugated diene production, reduce antioxidant reserve capacity, or change left ventricular functional recovery. In contrast, *uncontrolled cardiac reoxygenation* raised nitric oxide and conjugated diene production 19- and 13-fold, respectively ($p < 0.05$ vs control), reduced antioxidant reserve capacity 40%, and contractility recovered only 21% of control levels. After *controlled cardiac reoxygenation* at oxygen tension about 400 mm Hg with cardioplegic solution containing KCl only, nitric oxide and conjugated diene production rose 16- and 12-fold, respectively ($p < 0.05$ vs control), and contractility recovered only 43% ± 5%. Normoxemic (oxygen tension of about 100 mm Hg) controlled cardiac reoxygenation with the same solution reduced nitric oxide and conjugated diene production 85% and 71%, and contractile recovery rose to 55% ± 7% ($p < 0.05$ vs uncontrolled reoxygenation). In comparison, *controlled cardiac reoxygenation* with an oxygen tension

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of about 400 mm Hg hypocalcemic, alkalotic, glutamate/aspartate blood cardioplegic solution reduced nitric oxide and conjugated diene production 85% and 62%, respectively, and contractility recovered 63% ± 4% ($p < 0.05$ vs KCl only). Normoxemic delivery of this solution resulted in negligible nitric oxide and conjugated diene production and 83% ± 8% recovery of contractility ($p < 0.05$ vs all other groups). These data show correlation between nitric oxide production during initial reoxygenation and the extent of oxidant damage (i.e., conjugated diene production) and link functional recovery to suppression of excessive nitric oxide production and limitation of lipid peroxidation by the interaction of oxygen tension and cardioplegic composition during initial reoxygenation. (J THORAC CARDIOVASC SURG 1995;110:1274-86)

The pathophysiology of ischemic/reperfusion damage differs from that of hypoxemic/reoxygenation injury in the immature heart.[1, 2] Previous studies show that control of the initial conditions of reperfusion and composition of the reperfusate blood cardioplegic solution limits cardiac damage when blood supply is restored after ischemia.[3, 4] We have documented that a similar blood cardioplegic strategy limits oxidant damage in the setting of hypoxemia/reoxygenation.[5, 6] It is now recognized that myocardial damage develops when molecular oxygen is reintroduced when extracorporeal circulation is started in hypoxemic hearts, and (1) is oxygen tension (Po_2) dependent,[7, 8] (2) occurs whether reoxygenation is gradual or sudden,[7, 9] (3) can be limited by delaying reoxygenation until the aorta is clamped and blood cardioplegia is delivered shortly after starting cardiopulmonary bypass (CPB),[6, 7] and (4) is caused in part by production of reactive oxygen intermediates via the heretofore unrecognized L-arginine–nitric oxide (·NO) pathway.[10-12]

Several cardioplegic components that are cardioprotective for both reperfusion and reoxygenation damage also limit ·NO production; constitutive ·NO synthase activity is Po_2 calcium-calmodulin dependent,[13] its activity is down-regulated by alkalosis and the reduced shear stress accompanying gentle reperfusion pressure,[14] and glutamate and aspartate limit L-arginine transport.[15-18]

This study was undertaken in cyanotic hearts undergoing *controlled cardiac reoxygenation*, whereby CPB was initiated at the ambient hypoxemic Po_2 and reoxygenation was delayed until the cardioplegic solution was administered. The intent was to distinguish the interaction between Po_2 and cardioplegic constituents in limiting ·NO synthase activity (hypocalcemia, alkalosis, and glutamate/aspartate). The results show that avoiding excessive ·NO production correlates with decreased oxidant damage and offsets the contractile impairment that otherwise follows *uncontrolled cardiac reoxygenation*.

Material and methods

Experimental model. Thirty-five 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). The surgical preparation, including vessel cannulation for CPB and placement for monitoring and blood sampling, was similar to that reported previously.[19]

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 the 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 (SVRI), pulmonary vascular resistance index (PVRI), and left ventricular stroke work index (LV-SWI) were calculated with the following equations:

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

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

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

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

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.

Myocardial performance. LV pressure and conductance catheter signals were amplified and digitalized to inscribe LV pressure-volume loops after correction for tissue conductance.[20] The series of pressure-volume loops were generated under varying loading conditions by transient occlusion of the inferior vena cava during a 7-second period of apnea. Measurements were made before hypoxemia (control) and 30 minutes after discontinuation of CPB. The end-systolic pressure volume relationship (ESPVR) was analyzed by an interactive videographics data analysis computer program (Spectrum, Bowman Gray School of Medicine, Winston-Salem, N.C.) on an 386/33 MHz IBM computer (IBM, Armonk, N.Y.), and LV performance was described as the slope of linear regression (end-systolic elastance [Ees]), as described previously.[21, 22] Myocardial function before and after CPB was also evaluated with Starling curves by infusing CPB blood into the inferior vena cava at 5 ml/min/kg while recording CO, MAP, and LAP.

Myocardial ·NO production. ·NO was determined in plasma by reconverting its oxidation *N*-products nitrite (NO_2^-) and nitrate (NO_3^-), to ·NO in measuring chemiluminescence after ·NO reacts with ozone using nitrogen oxide analyzer (Model 2108, DASIBI Environmental Corporation, Glendale, Calif.).[23] Blood samples were taken from the coronary perfusate (blood cardioplegia) and coronary effluent (coronary sinus blood) to assess arteriovenous differences in ·NO during induction of blood cardioplegia. Production of ·NO is measured by the ·NO production = CBF × (V − A)/100 gm heart weight where A and V are the respective concentrations of ·NO measured in the aortic and coronary sinus blood during cardioplegic delivery when the cardioplegic solution was administered in a fixed coronary blood flow rate (CBF) by calibrated roller pump as described under control studies.

Myocardial oxidant injury

Myocardial conjugated diene production. Additional aliquots of coronary artery perfusate (blood cardioplegia) and coronary venous effluent were sampled to determine conjugated diene production. Samples were centrifuged immediately for 5 minutes at 1000g. Plasma was stored in liquid nitrogen for later analysis of conjugated dienes as described previously.[24] Conjugated diene production was calculated by the formula: CBF × (V − A)/100 gm heart weight where A and V are the concentrations of conjugated dienes in the blood cardioplegic perfusate and coronary sinus effluent respectively and CBF is the coronary blood flow rate in milliliters per minute.

Antioxidant reserve capacity. The myocardial antioxidant state was assessed by determining in vitro lipid peroxidation in cardiac tissue that was homogenized and incubated with *t*-butylhydroperoxide at concentrations varying from 0 to 4 mmol/L for 30 minutes at 37° C by the method of Godin and coworkers[25] described previously.

Experimental groups

Nonhypoxemic studies

BLOOD CARDIOPLEGIA WITHOUT HYPOXEMIA. Six normoxemic piglets (Po_2 of about 100 mm Hg) underwent 60 minutes of hyperoxemic CPB at a Po_2 of about 400 mm Hg without preceding hypoxemia. After 5 minutes of CPB, they underwent 30 minutes of aortic clamping with a hyperoxemic blood cardioplegic solution that was developed experimentally[26-28] and is used clinically.[29, 30] The blood cardioplegic management protocol included warm cardioplegia (37° C) for 3 minutes followed by cold cardioplegia for 2 minutes (4° C) and a 3-minute warm infusion just before aortic unclamping. Infusion rate was regulated initially by calibrated pump at 10 ml/min/kg to produce arrest and 5 ml/min/kg thereafter with an aortic pressure averaging about 50 mm Hg. CPB was discontinued 25 minutes after aortic unclamping, and functional and biochemical measurement were made 30 minutes later. This CPB protocol was followed in all subsequent studies when hypoxemic piglets were reoxygenated on CPB.

Hypoxemia/reoxygenation studies

Twenty-nine piglets were made hypoxemic by lowering inspired O_2 to 6% to 7% to reduce Po_2 to about 25 mm Hg for up to 120 minutes before reoxygenation on CPB for 60 minutes. Extracorporeal circulation was begun before 120 minutes if mean arterial pressure fell below 30 mm Hg or arterial pH could not be kept above 7.35 by infusing $NaHCO_3$ (1 mEq/kg) by bolus injection.

UNCONTROLLED CARDIAC REOXYGENATION. Nine piglets were reoxygenated by the conventional clinical procedure of starting CPB at Po_2 about 400 mm Hg and underwent a 30-minute interval of aortic clamping with the use of the aforementioned blood cardioplegic protocol.

CONTROLLED CARDIAC REOXGYENATION. Twenty hypoxemic piglets were placed on CPB using a hypoxemic Po_2 of about 25 mm Hg prime in the CPB circuit to match the pre-CPB Po_2. This level of hypoxemia was produced by adjusting the blend of N_2 and O_2 in the CPB circuit. All underwent *controlled cardiac reoxygenation*, whereby the increase in Po_2 in the CPB circuit and blood cardioplegic solution was *delayed* until the aorta was clamped and blood cardioplegia was given 5 minutes after CPB was started. All piglets underwent 30 minutes of aortic clamping using the aforementioned blood cardioplegic and CPB regimen. The Po_2 in the CPB and blood cardioplegic solution and blood cardioplegic formulation were adjusted as described below.

Blood cardioplegia with KCl only

NORMOXEMIC REOXYGENATION. Five piglets received normoxemic blood cardioplegia (Po_2 about 100 mm Hg) containing only KCl (30 mEq/L).

HYPEROXEMIC REOXYGENATION. Five other piglets received hyperoxemic cardioplegia (Po_2 about 400 mm Hg) containing only KCl (30 mEq/L).

Blood cardioplegia with glutamate/aspartate, hypocalcemia, and alkalosis

NORMOXEMIC REOXYGENATION. Five piglets received normoexemic (Po_2 about 100 mm Hg) blood cardioplegia containing the hypocalcemic, alkalotic, glutamate/aspartate additives used in control studies.

HYPEROXEMIC REOXYGENATION. Five piglets received a hyperoxemic (Po_2 about 400 mm Hg) blood cardioplegic solution containing the aforementioned additives.

Arterial Po_2 was raised to about 400 mm Hg immediately after aortic unclamping in piglets undergoing nor-

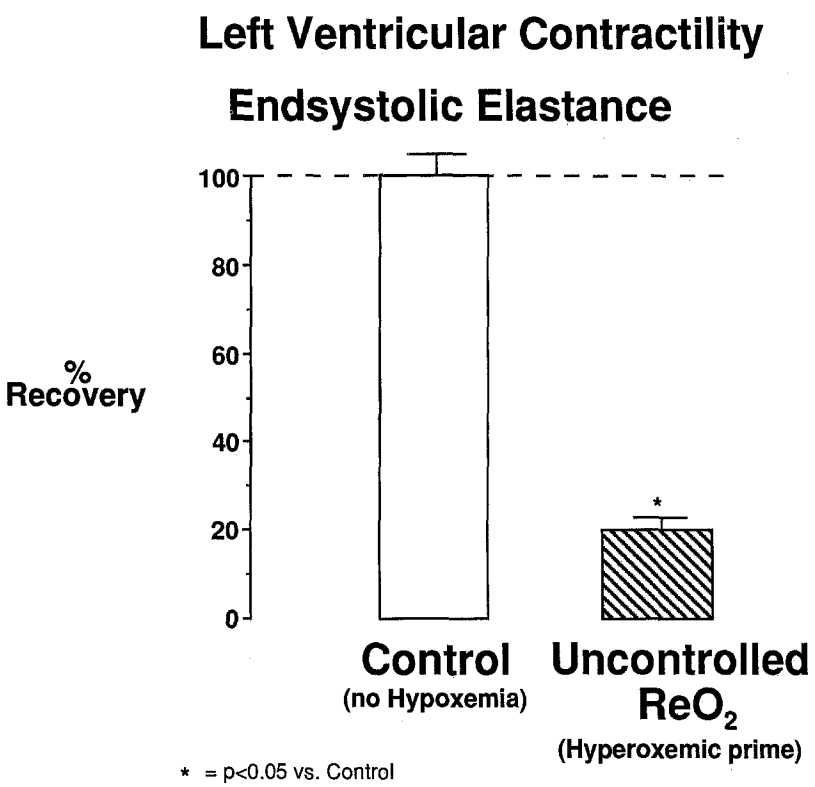

Fig. 1. Percent of recovery of Ees in control studies with CPB without hypoxemia (no hypoxemia) and after uncontrolled reoxygenation *(ReO_2)*.

moxemic reoxygenation. Mean arterial blood pressure was adjusted to 60 to 70 mm Hg after cardiac contraction resumed. Arterial blood gases and electrolytes were restored to normal levels before final measurements of biochemical variables and contractile function were made 30 minutes after CPB. Hearts were then arrested with cold (4° C) blood cardioplegia (KCl 30 mEq/L) to stop metabolism, and a transmural LV biopsy was obtained (about 200 mg of tissue). The subendocardial portion was separated, frozen quickly in liquid nitrogen, and stored for biochemical analyses.

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 (conjugated dienes) 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. Hypoxemia caused tachycardia (heart rate increased from 170 ± 10 to 206 ± 15 beats/min), pulmonary vasoconstriction (PVRI increased 120% ± 21%), peripheral vasodilatation (SVRI) decreased 34% ± 12%, and an initial increase in cardiac output occurred (28% ± 8%). There was no statistically significant difference in bolus $NaHCO_3$ injections (averaging 1 to 2 per piglet) or in the duration of hypoxemia among the groups, as ventilator hypoxemia was tolerated for an average of 75, 63, 65, 64, and 63 minutes, respectively.

All piglets could support the circulation after CPB was discontinued and achieved normal to near-normal cardiac output after volume infusion. A higher LAP was needed to maintain the same LVSWI after either uncontrolled reoxygenation or hyperoxemic controlled reoxygenation with KCl cardioplegia than after normoxemic reoxygenation; no further augmentation of stroke work index by volume infusion occurred after LAP was more than 8 to 10 mm Hg.

LV contractility. Myocardial performance was unaffected in nonhypoxemic piglets undergoing 1 hour of hyperoxemic CPB and blood cardioplegia. In contrast, *uncontrolled reoxygenation* with the same

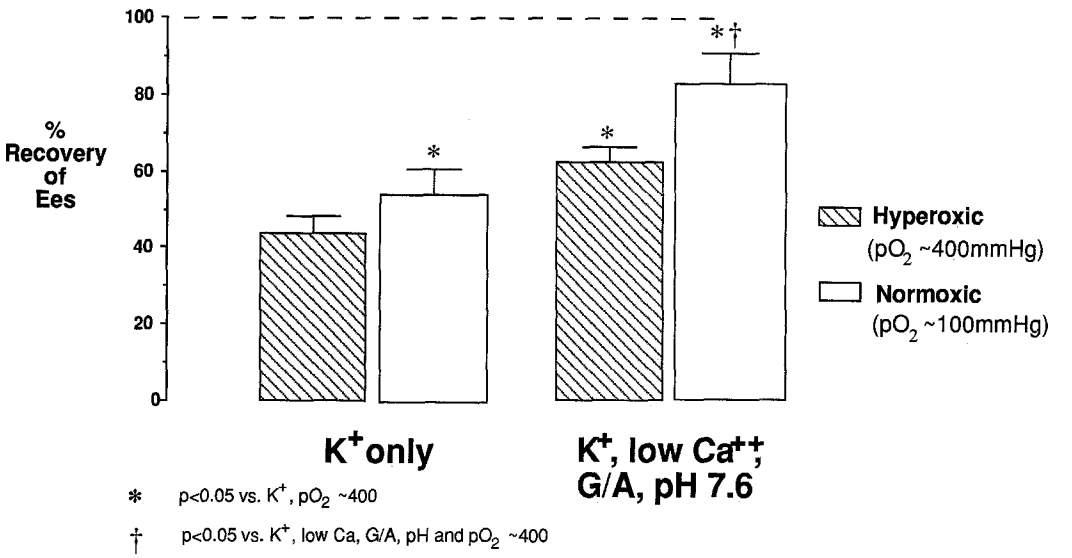

Fig. 2. Percent recovery of Ees after controlled cardiac reoxygenation with KCl blood cardioplegia *(KCl only)* or with blood cardioplegia containing low calcium *(low Ca^{++})*, glutamate/aspartate *(G/A)*, and alkalosis (pH 7.6).

CPB and blood cardioplegic solution was associated with only 21% recovery of LV Ees (Fig. 1). *Controlled cardiac reoxygenation* improved contractile recovery in all studies, with the extent of improvement related closely to the interaction between Po_2 and blood cardioplegic composition. LV Ees increased to 43% ± 5% when reoxygenation was delayed until Po_2 about 400 mm Hg blood cardioplegia with KCl only was used for myocardial management during aortic clamping, and rose to 55% ± 7% when Po_2 was kept about 100 mm Hg.

Further improvement to 63% ± 4% return of contractile function occurred when the Po_2 of about 400 mm Hg (Fig. 2) cardioplegic solution contained glutamate/aspartate, hypocalcemia, and alkalosis, and rose to 83% ± 8% at Po_2 of about 100 mm Hg ($p < 0.05$ vs KCl only).

Biochemistry results

Nitric oxide production. Uncontrolled cardiac reoxygenation with the CPB circuit primed with blood at Po_2 of about 400 mm Hg caused a 19-fold increase in ·NO production compared with control nonhypoxemic piglets given the same hyperoxemic blood cardioplegic solution ($p < 0.05$) (Fig. 3). A similarly pronounced ·NO production (16-fold increase, $p < 0.05$) occurred after controlled cardiac reoxygenation when the blood cardioplegic solution was delivered at Po_2 about 400 mm Hg and contained only KCl (Fig. 4). In contrast, ·NO increased only threefold when the KCl blood cardioplegic solution was made normoxemic ($p < 0.05$ vs uncontrolled reoxygenation). Supplementation of the blood cardioplegic formulation with glutamate/aspartate, hypocalcemia, and alkalosis caused only a twofold increase in ·NO production despite Po_2 of about 400 mm Hg, and ·NO production was comparable with control values when this cardioplegic solution was delivered at a Po_2 of about 100 mm Hg ($p < 0.05$ vs uncontrolled reoxygenation).

Conjugated diene production. The aforementioned changes in ·NO production were paralleled by changes in conjugated dienes, because *uncontrolled cardiac reoxygenation* caused a 13-fold increase in conjugated diene levels (from 3 ± 2 to 42 ± 4 A233 nm/min/100 g) (Fig. 5), which was unchanged by *controlled cardiac reoxygenation* when the Po_2 about 400 mm Hg cardioplegic solution contained KCl only (39 ± 11 A233 nm/min/100 g, $p > 0.05$ vs control). Conjugated diene production was reduced 71% (to 12 ± 6) when the KCl cardioplegic solution was delivered normoxemically at Po_2 about 100 mm Hg ($p < 0.05$ vs uncontrolled reoxygenation). A comparable reduction (62%) in conjugated diene levels occurred despite a Po_2 of about 400 mm Hg reoxygenation when the cardioplegic formulation contained constituents that inhibit NO synthase (16 ± 8 nmol, $p < 0.05$ vs uncontrolled reoxygenation); Normoxemic delivery

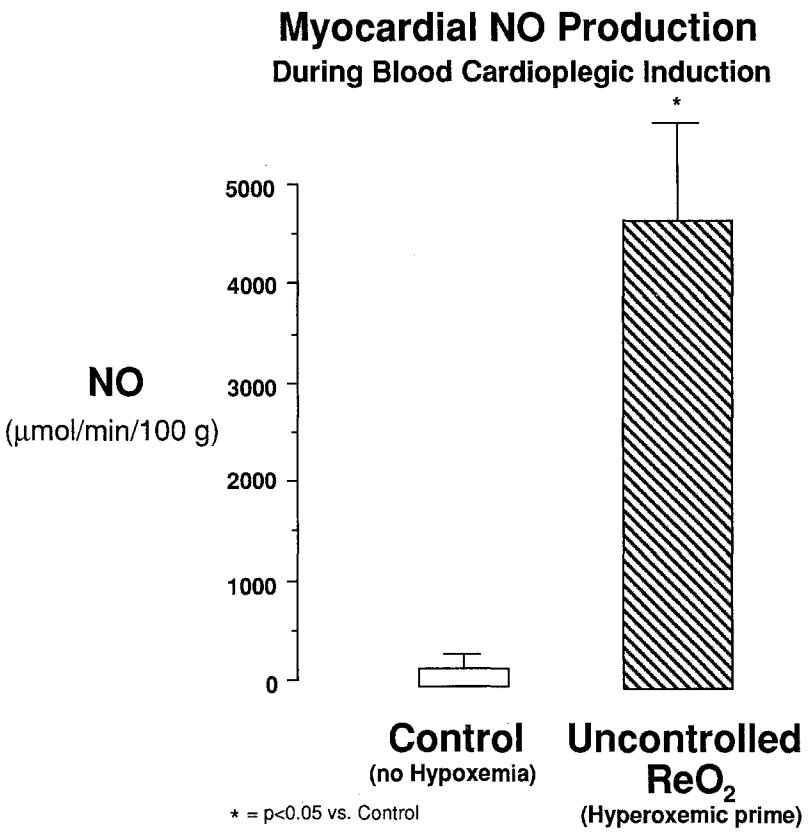

Fig. 3. Myocardial ·NO production during blood cardioplegic induction in control studies of CPB without hypoxemia and after uncontrolled reoxygenation (ReO_2).

of this formulation resulted in conjugated diene production that was comparable with nonhypoxemic piglets (Fig. 6, $p < 0.05$).

Antioxidant reserve capacity. Uncontrolled cardiac reoxygenation reduced antioxidant reserve capacity (AORC) 40% (compared with control animals without hypoxemia, $p < 0.05$). Mild but insignificant reductions in AORC occurred also when controlled cardiac reoxygenation was managed with blood cardioplegic solution containing KCl only, because AORC was reduced 18% and 17%, respectively, with hyperoxemic and normoxemic management (compared with the control group). Controlled cardiac reoxygenation with hyperoxemic blood cardioplegia containing potential ·NO synthase inhibitors was also associated with a 17% reduction in AORC compared with the control group, whereas normoxemic management at Po_2 about 100 mm Hg with the same solution maintained normal AORC values and improved AORC 36% compared with uncontrolled reoxygenation ($p < 0.05$).

Discussion

This study provides further evidence that reoxygenation injury causes dysfunction after CPB and emphasizes a linkage between the interaction of Po_2 and blood cardioplegia composition in affecting ·NO production and biochemical and functional evidence of oxidant damage. These data confirm the potential cytotoxic role of ·NO reported by others.[12, 31-33] The detrimental effects of reoxygenation nullified the cardioprotective action of an otherwise safe blood cardioplegic myocardial management protocol, and damage was limited when reintroduction of molecular oxygen was delayed until the heart was given a normoxemic blood cardioplegic solution developed originally to treat reperfusion damage.[3] The effects of this strategy seemed in part related to how the control of Po_2 and blood cardioplegic composition altered the L-arginine–·NO pathway.

Role of NO. Recent findings confirm that ·NO may produce myocardial contractile dysfunction.[10, 31, 33] The present report quantifies a substan-

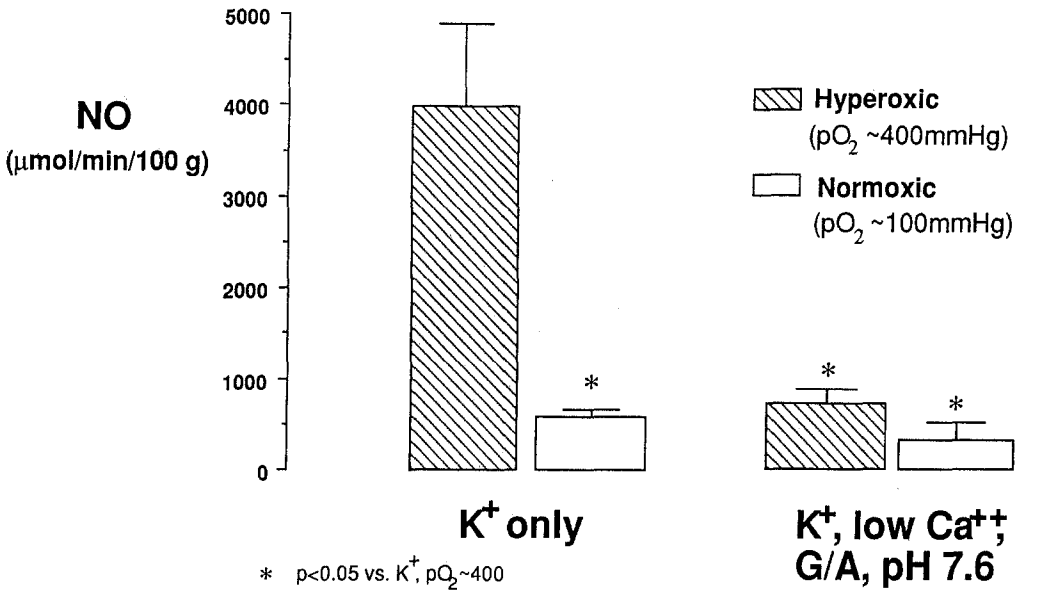

Fig. 4. Myocardial ·NO production during cardioplegic induction after controlled cardiac reoxygenation with KCl blood cardioplegia *(KCl only)* or with blood cardioplegia containing low calcium *(low Ca^{++})* glutamate/aspartate *(G/A),* and alkalosis (pH 7.6).

tially greater production of ·NO after reoxygenation than our previous report,[10] because we determined actual production rates of nitrite and nitrate rather than isolated serum nitrite levels alone. Myocardial ·NO production during reoxygenation correlated with the severity of oxidant injury because a suppression of ·NO production by controlling blood cardioplegic Po_2 composition reduced lipid peroxidation and improved myocardial function. Potential sources of ·NO include the endothelium, myocytes, endocardium, and mitochondria,[13, 34-36] but this study does not distinguish their relative contributions to the observed damage or examine the extent of suppression of ·NO production by individual blood cardioplegic solution elements.

Arginine is a biochemical precursor of ·NO that controls endothelial-derived relaxation. ·NO also inhibits vascular adherence of neutrophils and platelets, which perhaps accounts for the salutary effects of L-arginine in the ischemic/reperfusion model,[37] where blood flow is stagnant and neutrophils become adherent during ischemia and activated during reperfusion.[38] Conversely, L-arginine accelerates reoxygenation damage in the in vivo hypoxemic model in which CBF is high and neutrophil adherence is less likely.[10, 11] Clearly, a balance between the physiologic and pathologic effects of ·NO must be established. Furthermore, lactate accumulation may down-regulate metabolism during ischemia and provide reducing equivalents during reperfusion,[39] whereas the high coronary flow during hypoxemia prevents lactage buildup.

These in vivo findings are consistent with recent in vitro studies showing that the maximum velocity V_{max} of constitutive ·NO synthase is related to oxygen tension.[40] ·NO production rose markedly in hypoxemic hearts reoxygenated with a Po_2 of about 400 mm Hg blood cardioplegia with K+ only, whereas ·NO production and subsequent myocardial dysfunction were reduced markedly after controlled reoxygenation with a hyperoxemic cardioplegic solution with components that reduced ·NO synthase activity. Normal ·NO production, minimal impairment of AORC, and 83% functional recovery followed controlled reoxygenation with a Po_2 of about 100 mm Hg cardioplegic solution of similar composition. We speculate that the excess of ·NO reacts with superoxide anion (O_2^-) and generates the intermediate peroxynitrite ($OONO^-$), which decomposes to generate highly toxic O_2 and N_2 intermediates that cause lipid peroxidation.[41, 42]

Pronounced ·NO production after abrupt uncontrolled reoxygenation may result in adverse biochemical reactions besides those related to peroxynitrite formation. ·NO mediates iron release from ferritin, the protein that stores excess intracel-

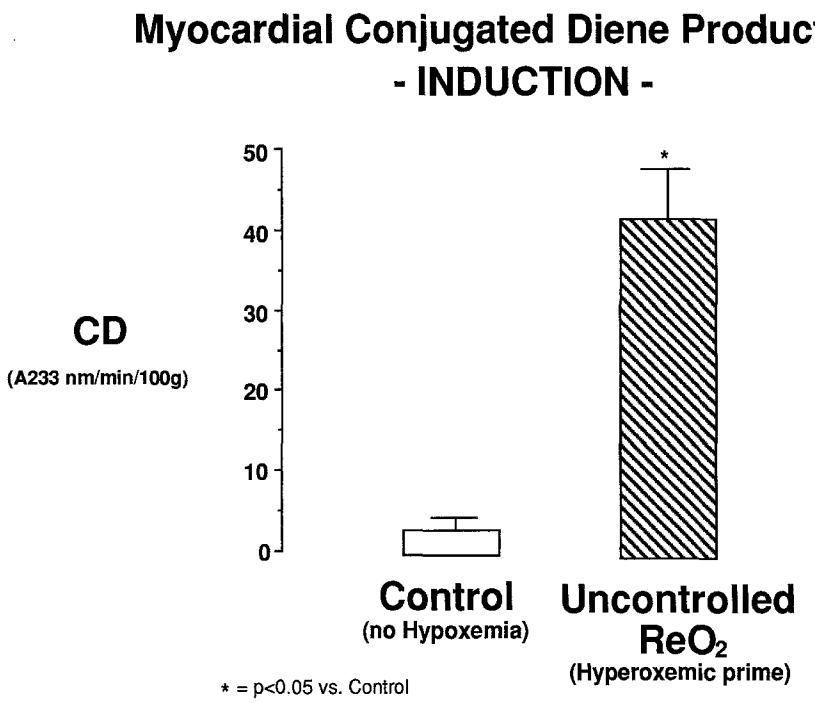

Fig. 5. Myocardial conjugated diene production during cardioplegic induction in control studies of CPB without hypoxemia, and after uncontrolled reoxygenation *(ReO_2)*.

lular iron,[43] so that ·NO may participate in the Haber-Weiss reaction by mechanisms that were not appreciated previously. This observation is underscored by studies showing that deferoxamine, an iron chelator, prevents reperfusion- and reoxygenation-related myocardial damage.[44-46]

These observations imply the possibility of an *·NO paradox* much like this O_2[8] and calcium paradox,[8] whereby a substance responsible for modulating several physiologic functions (i.e., vascular tone, white blood cell, and platelet adherence) may become deleterious when produced in high concentrations upon reoxygenation. The confusion over how the same substance can be both salutary and detrimental is resolved by recognizing that substances may have different actions *during versus after* reoxygenation.

Role of Po_2. CPB in normoxemic and hypoxemic infants is initiated usually at high Po_2 (about 400 mm Hg) without consideration of the possible cytotoxic effects of abrupt hyperoxemia. This study shows that only 5 minutes of uncontrolled reoxygenation by hyperoxemic CPB produced sufficient damage to nullify the benefits of blood cardioplegia. Free radical generation and subsequent myocardial injury after reoxygenation are proportionate to Po_2,[47] and normoxemic reoxygenation is neuroprotective in hypoxemic neuronal cells.[48] Gradual reoxygenation without blood cardioplegia reduces damage only negligibly when Po_2 is raised to Po_2 about 400 mm Hg over a 1-hour period.[7] We suspect that delaying reoxygenation avoided the burst of ·NO and O_2^- associated with abrupt reoxygenation, because CPB initiated at hypoxemic Po_2 about 25 mm Hg followed by reoxygenation with normoxemic blood cardioplegia resulted in negligible excessive ·NO production, avoided lipid peroxidation, and preserved AORC when the blood cardioplegic formulation down-regulated ·NO synthase activity. Controlled cardiac reoxygenation with blood cardioplegia containing KCl only produced only limited recovery. The importance of the interaction of Po_2 and blood cardioplegic composition was amplified by the finding that a Po_2 about 400 mm Hg blood cardioplegic formulation that down-regulated ·NO synthase activity reduced ·NO production and antioxidant injury *more* than a Po_2 about 100 mm Hg formulation containing KCl only (Figs. 3 through 6).

Controlled cardiac reoxygenation with blood cardioplegia. The substrate-enriched blood cardioplegic solution used in these studies was developed experimentally as a metabolic way to actively resus-

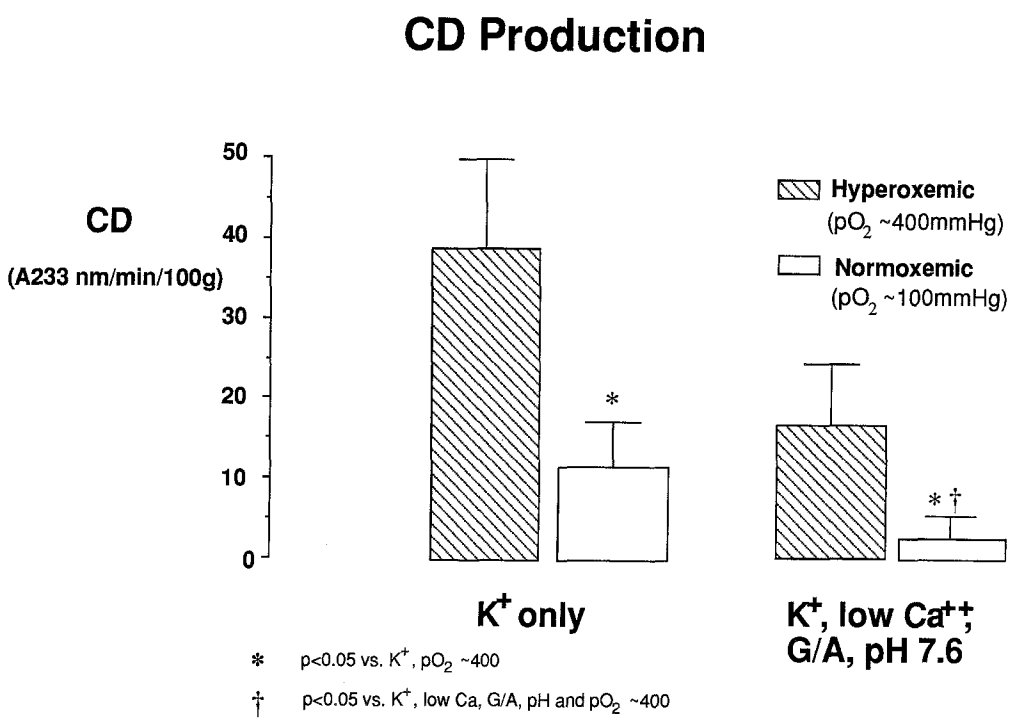

Fig. 6. Myocardial conjugated diene *(CD)* production during cardioplegic induction after controlled cardiac reoxygenation with KCl blood cardioplegia *(KCl only)* or blood cardioplegia containing low calcium *(low CA^{++})*, glutamate/aspartate *(G/A)*, and alkalosis (pH 7.6).

citate energy-depleted hearts[4, 49, 50] and has been used in high-risk adult and pediatric patients.[51, 52] This study confirms its safety in non-hypoxemic control piglets and substantiated that only 5 minutes of uncontrolled reoxygenation offsets its metabolic and functional benefits. Our protocols were cardiac directed and did not deal with reoxygenation damage reported to affect other organs (i.e., lung and liver).[39, 47, 53-55] The near-complete myocardial functional recovery after *controlled cardiac reoxygenation* is consistent with previous observations in which controlled reperfusion was used after regional and global ischemia.[3, 4, 30] We speculate that several aspects of controlled cardiac reoxygenation with blood cardioplegia limited reoxygenation injury by actions enumerated previously[3] and by some recently defined mechanisms described below.

Normothermic potassium arrest with hypocalcemic, alkalotic blood. The importance of initiating reoxygenation in the normothermically arrested state is underscored by the finding that reoxygenation hypercontraction and sarcolemmal disruption can be prevented by temporary contractile blockade.[56] This suggests that some of the limited energy produced during initial reoxygenation is used to normalize cytosolic Ca^{++}, presumably by improving the $Na^{+} - Ca^{++}$ exchange.[57] Functional recovery after controlled cardiac reoxygenation of blood cardioplegia containing KCl only was inferior to that achieved when NO synthase was down-regulated by the other formulation components (Fig. 4).

Recent studies link reoxygenation to intracellular calcium loading and membrane damage.[57] Hypocalcemic reoxygenation presumably decreases mitochondrial calcium loading and avoids consequent impairment of mitochondrial and energy production.[58] Hypocalcemia may also limit activation of phospholipases, which degrade membrane phospholipids and slow the release of reactive oxygen intermediates via the arachidonic and xanthine oxidase pathways. Constitutive ·NO synthase is Ca^{++}/calmodulin dependent, so that decreasing extracellular Ca^{++} attenuates activation of both endothelial and endocardial ·NO synthase and reduces ·NO production.[13, 36] Reperfusion alkalosis also favors inactivation of ·NO by catalyzing its oxidation to inorganic nitrate, because ·NO is most stable at acidotic pH.[59]

Glutamate/aspartate supplementation. Both hypoxemia and ischemia deplete cytosolic concentrations of key precursors of Krebs' cycle intermediates

(glutamate and aspartate), thus reducing their availability for optimal tricarboxylic acid function on reoxygenation.[60, 61] Their replenishment in previously hypoxemic or ischemic heart may restore near-normal function, presumably via improved energy metabolism.[62-64] Glutamate and aspartate may also affect the L-arginine–·NO pathway via their conversion to glutamine. Arginine uptake is essential for ·NO synthesis, and is regulated by the cationic amino acid transport system that can be inhibited by L-glutamine and neutral amino acids.[16, 17] Glutamine synthetase which is present in cardiac muscle, converts glutamic acid to L-glutamine,[17] and L-glutamine is synthesized from aspartate via aspartate amino transferase.[18] These observations imply that glutamate and aspartate may inhibit L-arginine transport and subsequent ·NO production via their conversion to L-glutamine, in addition to their effect on cardiac energy metabolism.

Reduced reperfusion pressure. Reducing coronary artery pressure during reperfusion improves endothelial-dependent function.[65] This was attributed to a reduction in mechanical damage to vulnerable endothelial cells, but endothelium-derived ·NO is linked closely to shear stress,[14] so that decreasing shear stress by gentle reperfusion may limit the ·NO that is generated.

Po_2 in blood cardioplegia. We showed previously that lowering Po_2 in the CPB circuit reduced reoxygenation injury minimally without blood cardioplegia if the end point was hyperoxemic at Po_2 about 400 mm Hg.[7] The present studies indicate that blood cardioplegic solution (Po_2) may influence the effectiveness of different factors in the blood cardioplegic strategy, because O_2^- and ·NO production are Po_2 dependent.[40, 47] Hyperoxemic blood cardioplegia is probably never needed, because normoxemia (Po_2 about 100 mm Hg) provides near complete O_2 saturation and O_2 demands are negligible in the arrested decompressed heart.[66] This concept is supported by the superior results obtained with normoxemic versus hyperoxemic blood cardioplegic management strategy with blood cardioplegic solutions containing either KCl only or with constituents that reduce ·NO synthase activity.

Clinical implications. These data suggest that "unintended" cardiac reoxygenation injury that follows conventional institution of hyperoxemic CPB can be reduced by delaying reoxygenation until the aorta is clamped and a blood cardioplegic solution is delivered that reduces ·NO overproduction and oxidant injury. The pre-CPB dysfunction that characterized this acutely hypoxemic model is rare in patients with compensated cyanosis, but its incidence was similar in all experimental groups. Recovery occurred *only* in piglets undergoing controlled cardiac reoxygenation and was proportionate to the reduction of ·NO production and oxidant damage. The extent of functional recovery may have been underestimated in all studies by the brief post-CPB observation period and raising Po_2 to 400 mm Hg after CPB was stopped.

Similar changes in antioxidants levels, lipid peroxidation, and functional depression are reported in cyanotic infants placed on CPB for cardiac repair.[7, 67-72] Reduced systolic function, which may progress to "cardiac stun," occurs without surgical ischemia[7, 73-74] in cyanotic infants who undergo extracorporeal membrane oxygenation for respiratory failure. All piglets could be weaned from CPB, but our findings of depressed function (exposed by testing functional reserve) may explain why inotropic support is often needed after surgery in cyanotic children.

Our results suggest that altering routine techniques of conventional CPB may limit reoxygenation damage. Initiation of CPB at the ambient hypoxemic Po_2 reduces ·NO production and would likely be safe because the flow rate in the extracorporeal circuit could be augmented to ensure O_2 delivery when bypass is started. Furthermore, normoxemic initiation of CPB at Po_2 of about 100 mm Hg ensures about 99% oxygen saturation without adjustment of CPB flow. It is unlikely that hyperoxemic CPB is ever needed intraoperatively or postoperatively because $Po_2 > 100$ to 150 mm Hg increases O_2 content negligibly. The methods of providing controlled cardiac reoxygenation used in this study are available in routine practice and can hopefully improve clinical outcome if our results are reproduced by others.

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