STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY WITH AORTIC CLAMPING:

XI. Cardiac advantages of normoxemic versus hyperoxemic management during cardiopulmonary bypass

The conventional way to start cardiopulmonary bypass is to prime the cardiopulmonary bypass circuit with hyperoxemic blood (oxygen tension about 400 mm Hg) and deliver cardioplegic solutions at similar oxygen tension levels. This study tests the hypothesis that an initial normoxemic oxygen tension strategy to decrease the oxygen tension-dependent rate of oxygen free radical production will, in concert with normoxemic blood cardioplegia, limit reoxygenation damage and make subsequent hyperoxemia (oxygen tension about 400 mm Hg) safer. Thirty-five immature (3 to 5 kg, 2 to 3 week old) piglets underwent 60 minutes of cardiopulmonary bypass. Eleven control studies at conventional hyperoxemic oxygen tension (about 400 mm Hg) included six piglets that also underwent 30 minutes of blood cardioplegic arrest. Of 25 studies in which piglets were subjected to up to 120 minutes of ventilator hypoxemia (reducing fraction of inspired oxygen to 5% to 7%; oxygen tension about 25 mm Hg), 11 underwent either abrupt (oxygen tension about 400 mm Hg, $n = 6$) or gradual (increasing oxygen tension from 100 to 400 mm Hg over a 1-hour period, $n = 5$) reoxygenation *without* blood cardioplegia. Fourteen others underwent 30 minutes of blood cardioplegic arrest during cardiopulmonary bypass. Of these, nine were reoxygenated at oxygen tension about 400 mm Hg, and five others underwent normoxemic cardiopulmonary bypass and blood cardioplegia (oxygen tension about 100 mm Hg) with systemic oxygen tension raised to 400 mm Hg after aortic unclamping. Measurements of lipid peroxidation (conjugated dienes and antioxidant reserve capacity) and contractile function (pressure-volume loops, conductance catheter, end-systolic elastance) were made before and during hypoxemia and 30 minutes after reoxygenation. Hyperoxemic cardiopulmonary bypass did not produce oxidant damage or reduce functional recovery after cardiopulmonary bypass in nonhypoxemic controls. In contrast, abrupt and gradual reoxygenation *without* blood cardioplegia produced significant lipid peroxidation (84% increase in conjugated dienes), lowered antioxidant reserve capacity $68\% \pm 5\%$, $44\% \pm 8\%$, respectively, and decreased functional recovery $75\% \pm 6\%$ ($p < 0.05$), $66\% \pm 4\%$ ($p < 0.05$). Similar impairment followed abrupt reoxygenation before blood cardioplegic myocardial management, because conjugated diene production increased 13-fold, antioxidant reserve capacity fell 40%, and contractility recovered only $21\% \pm 2\%$ ($p < 0.05$). Conversely, normoxemic induction of cardiopulmonary bypass and blood cardioplegic myocardial management reduced conjugated diene production 73%, avoided impairment of antioxidant reserve capacity, and resulted in $58\% \pm 11\%$ recovery of contractile function. We conclude that these data suggest that reoxygenation damage is (1) oxygen tension dependent,

Kai Ihnken, MD, Kiyozo Morita, MD, and Gerald D. Buckberg, MD, and
Helen H. Young, PhD, *Los Angeles, Calif.*

From the Division of Cardiothoracic Surgery, Department of
Surgery, the University of California at Los Angeles School of
Medicine, Los Angeles, Calif.

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Address for reprints: Gerald D. Buckberg, MD, Department of
Surgery, B2-375 CHS, UCLA Medical Center, P.O. Box
951741, 10833 Le Conte Ave., Los Angeles, CA 90095-1741.

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(2) occurs whether reoxygenation is abrupt or gradual in the absence of blood cardioplegia, and (3) can be reduced by normoxemic management when CPB is started and during blood cardioplegic administration before subsequent reintroduction of hyperoxemia. (J THORAC CARDIOVASC SURG 1995;110:1255-64)

Reintroduction of molecular oxygen in hypoxemic hearts during initiation of cardiopulmonary bypass (CPB) produces a reoxygenation injury¹⁻⁴ that may nullify an otherwise safe myocardial management technique of blood cardioplegia during aortic clamping.^{5,6} The role of oxygen free radicals in this process is reinforced by studies showing that antioxidant interventions that interfere with relevant pathways of oxygen-mediated damage^{2,7-9} reduce lipid peroxidation, enzyme release^{3,9-12} and improve functional recovery after CPB.

The conventional way to start CPB is to produce hyperoxemia by mixing the patient's blood with the extracorporeal circuit primed at oxygen tension (P_{O_2}) about 400 mm Hg despite in vitro evidence of the P_{O_2} -dependent nature of reoxygenation damage,^{11,13,14} and in vivo findings that initial hyperoxemic CPB may produce damage before aortic clamping is imposed for surgical correction.^{4,15,16} We consider reoxygenation damage a biologic phenomenon that may affect other organs, because lung opacification follows hyperoxemic extracorporeal membrane oxygenation in cyanotic infants with pulmonary disorders¹⁷ and causes myocardial dysfunction,^{18,19} and reduced P_{O_2} during reoxygenation is neuroprotective to chronically hypoxemic neuronal cells.²⁰ This latter observation, coupled with the P_{O_2} -dependent extent of myocardial damage during reoxygenation,¹³ led to the current study that applies a similar strategy to hypoxemic immature hearts reoxygenated on CPB. Our findings confirm the occurrence of reoxygenation injury and show (1) it is not avoided by only slowing the rate of reoxygenation, and (2) its nullification of the cardioprotective effects of blood cardioplegic myocardial management can be limited by starting CPB and delivering blood cardioplegia at normoxemic (P_{O_2} about 100 mm Hg) levels before raising P_{O_2} to about 400 mm Hg, the conventional CPB management method.

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 catheter placement for monitoring and blood sampling, is similar to that reported previously.¹

Physiologic and biochemical determinations. Arterial blood gas, electrolyte, and hemoglobin measurements were made every 15 minutes and analyzed by a blood gas system (Blood Gas System 288, Ciba-Corning, Medfield, Mass.). Left ventricular (LV) performance was evaluated by inscribing end-systolic pressure volume relationship with a LV five-electrode conductance catheter with a distance of 4 mm between electrodes (Webster Laboratories, Baldwin Park, Calif.), inserted through the LV apex, connected to a Sigma 5-DF Signal Conditioner Processor (Leycom, Oegstgeest, The Netherlands). A series of declining LV pressure-dimension loops were obtained by inferior vena cava occlusion during a 7-second apnea period as described previously. End-systolic pressure volume relationship was analyzed by an interactive videographics data analysis computer program (Spectrum²¹) on a 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. Postbypass LV contractility was assessed by percent recovery of prebypass control value (percent Ees). Data were expressed as Ees.

Myocardial oxidant injury

Conjugated diene. Plasma conjugated dienes (CD) levels were measured in 0.5 ml plasma samples of arterial and coronary sinus blood to determine lipid peroxidation by the method of Lesnefsky and coworkers²² as discussed previously. Myocardial CD production was determined in studies in which blood cardioplegia was delivered from the following equation: CD production = CBF $\times$ V - A, where CBF is the cardioplegic flow rate delivered by calibrated roller pump and A and V are arterial and coronary sinus CDs.

Antioxidant reserve capacity. Cardiac vulnerability to oxidant stress was evaluated by incubating myocardial homogenates in varying concentrations (1 to 4 mmol/L) of the oxidant *t*-butylhydroperoxide and measuring malondialdehyde elution by the method of Godin et al.²³ as previously described.

Hemodynamics. Measurements of cardiac output (thermodilution), arterial pressure, and left atrial pressure were made before the start of hypoxemia (control), each 15 minutes during hypoxemia, and 15 and 30 minutes after

CPB was discontinued. Measurements were used to calculate left ventricular stroke work index as described previously.^{1, 24} At the conclusion of each experiment each heart was examined postmortem to ensure closure of the ductus arteriosus and foramen ovale.

Experimental groups

Nonhypoxemic studies. Eleven piglets underwent 60 minutes of CPB at PO_2 about 400 mm Hg. Six underwent 30 minutes of blood cardioplegic arrest according to the following protocol: after 5 minutes of CPB, they underwent 30 minutes of aortic clamping with a hyperoxemic blood cardioplegic solution that was developed experimentally²⁵⁻²⁷ and is used clinically.^{28, 29} 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 a calibrated pump at 10 ml/min/kg to produce arrest and 5 ml/min/kg thereafter with 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.

Hypoxemia on CPB studies. Twenty-four piglets underwent up to 2 hours of hypoxemia by reducing ventilator fraction of inspired oxygen (FiO_2) to 6% to 7% to lower arterial PO_2 to about 25 mm Hg. Extracorporeal circulation was started before 120 minutes if mean arterial pressure could not be kept above 30 mm Hg by volume infusion or pH above 7.3 by up to three bolus injections of sodium bicarbonate.

REOXYGENATION WITHOUT CPB

1. **Abrupt hyperoxemic reoxygenation.** Five piglets underwent reoxygenation by initiating CPB at PO_2 about 400 mm Hg and did not undergo aortic clamping.
2. **Gradual reoxygenation.** In five other piglets, CPB was started at PO_2 about 100 mm Hg, and PO_2 was raised to about 400 mm Hg by 100 mm Hg increments each 15 minutes during the 60 minutes of CPB. No piglet underwent an interval of aortic clamping.

REOXYGENATION WITH CPB STUDIES

In 14 piglets the aorta was clamped 5 minutes after the start of CPB using the blood cardioplegia management protocol described previously with the following PO_2 management method.

1. **Hyperoxemic management.** In nine piglets, CPB was started at PO_2 about 400 mm Hg, and blood cardioplegia was delivered at the same PO_2 .
2. **Normoxemic management.** In five piglets CPB was started at PO_2 about 100 mm Hg (as described previously), and PO_2 was maintained at 100 mm Hg in the blood cardioplegic management protocol.

In all piglets PO_2 was raised to 400 mm Hg immediately after aortic unclamping and kept at that level for the remainder of the study. Final hemodynamic and blood measurements were made 30 minutes after the discontinuation of bypass, after which myocardial biopsy samples were obtained for subsequent analyses.

Statistics. Data were analyzed with the use of the StatView V2.0 program (Abacus Concepts Inc., Berkeley,

Fig. 1. Percent recovery of LV Ees after abrupt or gradual reoxygenation *without* blood cardioplegia (see text for description).

Calif.) on a Macintosh IIfx 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

Systemic hemodynamics are shown in the Table and remained unchanged from control levels in nonhypoxemic piglets who underwent 60 minutes of CPB with or without 30 minutes of blood cardioplegic arrest. Hypoxemia produced tachycardia (heart rate rose from 170 ± 10 to 206 ± 15 beats/min) and initial increase in cardiac output ($35\% \pm 8\%$ at 30 minutes) and systemic vasodilation (systemic vascular resistance fell $34\% \pm 12\%$) and pulmonary vasoconstriction (pulmonary vascular resistance rose $120\% \pm 12\%$). Hemodynamic deterioration occurred in all piglets after approximately 60 minutes of hypoxemia requiring initiation of CPB before 2 hours in 17 of 24 piglets. The average hypoxemic duration was 78 ± 12 minutes, with a comparable number of piglets in each group needing premature CPB before 120 minutes.

Recovery of LV contractility (Ees) was complete in all nonhypoxemic control studies of piglets sub-

Table. Hemodynamic measurements

Variable	Control		ReO ₂ without BCP		ReO ₂ + BCP	
	CPB	BCP	Abrupt	Gradual	PO ₂ ~400	PO ₂ ~100
Hypoxia (min)	—	—	84 ± 9	60 ± 7	75 ± 8	65 ± 5
ReO ₂ <120 min	—	—	3/5	4/5	6/9	4/5
Mean aortic pressure (mm Hg)						
Control	70 ± 5	52 ± 5	69 ± 5	50 ± 3	63 ± 6	50 ± 2
End hypoxia	—	—	47 ± 6	50 ± 12	44 ± 7	39 ± 8
30 min after CPB	59 ± 4	49 ± 4	51 ± 5	56 ± 14	41 ± 4*	45 ± 8
Heart rate (beats/min)						
Control	170 ± 10	166 ± 9	170 ± 8	178 ± 13	202 ± 5	184 ± 5
End hypoxia	—	—	176 ± 7	173 ± 15	184 ± 18	170 ± 12
30 min after CPB	205 ± 15	205 ± 10	163 ± 8	170 ± 12	196 ± 20	176 ± 13
Cardiac index (ml/min/kg)						
Control	114 ± 18	74 ± 19	119 ± 9	129 ± 15	118 ± 6	98 ± 16
End hypoxia	—	—	93 ± 11	55 ± 15*	79 ± 19*	38 ± 26*
30 min after CPB	82 ± 8	64 ± 4	65 ± 7*	107 ± 20	85 ± 11	75 ± 9
SVRI (mm Hg · L ⁻¹ · kg)						
Control	676 ± 117	634 ± 84	616 ± 13	455 ± 82	537 ± 44	559 ± 82
End hypoxia	—	—	482 ± 50	356 ± 117	348 ± 62	444 ± 79
30 min after CPB	729 ± 55	828 ± 125	685 ± 98	513 ± 82	571 ± 89	715 ± 89
PVRI (mm Hg · min · L ⁻¹ · kg)						
Control	88 ± 13	101 ± 23	117 ± 32	92 ± 12	72 ± 8	107 ± 22
30 min after CPB	207 ± 30	212 ± 32	296 ± 34*	205 ± 48	312 ± 53*	177 ± 13

BCP, Blood cardioplegia; ReO₂, reoxygenation; control, no hypoxia; SVRI, systemic vascular index; PVRI, pulmonary vascular resistance index.**p* < 0.05 vs control.

jected to 60 minutes of CPB at PO₂ about 400 mm Hg with or without 30 minutes of aortic clamping with the blood cardioplegic myocardial management protocol. In contrast, recovery of Ees was 25% ± 5% after abrupt hyperoxemic reoxygenation and was not improved appreciably (34% ± 4% recovery of Ees) when PO₂ was raised from 100 mm Hg to 400 mm Hg gradually during the 60 minutes interval of CPB (*p* < 0.05 vs control, Fig. 1).

Initial hyperoxemic CPB negated the cardioprotective effects of blood cardioplegia, because Ees recovered only 21% ± 2% (Fig. 2) in piglets subjected to 30 minutes of aortic clamping after CPB was started at PO₂ about 400 mm Hg. Improved LV recovery occurred when PO₂ was kept at normoxemic levels (PO₂ about 100 mm Hg) during both initiation of CPB and while blood cardioplegia was delivered, as Ees returned to 58% ± 11% of prehypoxemic levels (PO₂ < 0.05 vs abrupt and gradual reoxygenation or hyperoxemic CPB and blood cardioplegia). However, final values for Ees did not return to prehypoxemic levels, and remained lower than after hyperoxemic CPB (with and without blood cardioplegia) in piglets that did *not* undergo pre-CPB hypoxemia.

Biochemical. Hyperoxemic CPB without preceding hypoxemia did not cause myocardial lipid per-

oxidation or reduce cardiac antioxidant reserve capacity, because no CDs were produced either during induction or reperfusion during the 30-minute interval of aortic clamping, and antioxidant reserve capacity was unchanged from control values after the 30-minute observation period after CPB. Myocardial cardiac CD production was evaluated only in piglets receiving blood cardioplegia, because coronary flow was known from the cardioplegic flow rate. Hyperoxemic blood cardioplegia caused a 12-fold increase in CD production compared with normoxemic controls (Fig. 3, A), and this was reduced by 73% when normoxemic strategy was used to start CPB and deliver blood cardioplegia in previously hypoxemic piglets. Myocardial CD production persisted throughout the aortic clamping interval in hearts managed by the hyperoxemic strategy, while no significant lipid peroxidation was evident during reperfusion when the blood cardioplegic solution was kept normoxemic (Fig. 3, B). Signs of lipid peroxidation continued after aortic unclamping after hyperoxemic reoxygenation, because coronary sinus CDs remained increased (Fig. 4) and did not return to control levels until CPB was discontinued. Conversely, CD levels fell to control levels during reperfusion after normoxemic manage-

* = $p < 0.05$ vs. Control
** = $p < 0.05$ vs. ReO₂ @ pO₂ ~ 400 mmHg

Fig. 2. Percent recovery of LV Ees in nonhypoxemic piglets undergoing hyperoxemic blood cardioplegia (PO₂) about 400 mm Hg and after reoxygenation of hypoxemic piglets with either normoxemic (PO₂ about 100 mm Hg) or hyperoxemic (PO₂ about 400 mm Hg) blood cardioplegic solution (see text for description).

ment and coronary sinus CDs did not increase after aortic unclamping.

Hyperoxemic reoxygenation produced a 40% reduction in antioxidant reserve when myocardial homogenates were incubated in the oxidant *t*-butylhydroperoxide (Fig. 5), whereas this impairment of antioxidant reserve capacity did not occur after the normoxemic reoxygenation strategy managing CPB and blood cardioplegia.

Discussion

This in vivo study of hypoxemia/reoxygenation in the immature piglet provides data to suggest that conventional clinical methods of starting CPB at high PO₂ produces cardiac reoxygenation damage that nullifies the cardioprotective effects of an otherwise safe myocardial management technique of blood cardioplegia.^{5,6} New information includes the finding that (1) the signs of lipid peroxidation, impaired antioxidant reserve capacity, and contractile recovery that follow abrupt reoxygenation are not avoided by more gradual reoxygenation (i.e., over 1 hour), (2) a blood cardioplegic strategy that is safe in immature porcine hearts⁵ and that limits

reperfusion damage is rendered ineffective by a brief (i.e., 5 minutes) interval of hyperoxemic reoxygenation, and (3) damage may be limited substantially by using a normoxemic PO₂ management strategy during initiation of CPB and blood cardioplegia delivery before raising PO₂ to the conventional hyperoxemic levels used during most cardiac operations on immature and adult hearts. Studies were terminated 30 minutes after CPB, so that the experimental design precluded assessment of reversibility or progression of the observed changes.

The role of oxidant damage in the genesis of biochemical and functional impairment after reoxygenation in this study seemed independent of the effects of CPB per se, because hyperoxemic CPB and blood cardioplegia did not produce CD production (the selected marker for lipid peroxidation), reduce antioxidant reserve capacity (the bioassay marker of endogenous antioxidant levels), or impair contractile recovery. Our findings of reoxygenation damage in the in vivo piglet model (which parallels more closely the human condition³⁰) are consistent with the in vitro findings that: (1) hypoxemia depletes endogenous antioxidants,^{10,31} which become further

Fig. 3. Myocardial CD production during cardioplegic induction and reperfusion in control piglets undergoing hyperoxemic (Po₂ 400 mm Hg) CPB and blood cardioplegia and in hypoxemic piglets reoxygenated with either normoxemic (Po₂ 100 mm Hg) or hyperoxemic (Po₂ 400 mm Hg) blood cardioplegic solution (see text for description).

exhausted with reoxygenation,³¹ and (2) a burst of oxygen free radicals follows reoxygenation that is associated with signs of lipid peroxidation is linked to

enzyme release, ultrastructural damage,^{10, 11} and functional depression.¹² Our data suggest that the impaired antioxidant reserve capacity that follows either abrupt

Conjugated Dienes (Coronary Sinus Blood)

Fig. 4. CD concentration in coronary sinus blood sampled during hypoxemia and reoxygenation with cardioplegia (BCP) on CPB. Control piglets did not undergo hypoxemia or reoxygenation, and hypoxemic piglets were reoxygenated with either normoxemic (Po₂ 100 mm Hg) or hyperoxemic (Po₂ 400 mm Hg) blood cardioplegic solution (see text for description).

or gradual reoxygenation renders the previously hypoxemic myocardium more vulnerable to ischemic/reperfusion damage during aortic clamping, as shown by Silverman and coworkers³² and Fujiwawa and colleagues.³³

The Po₂-dependent nature of this damage can be inferred from the experimental studies of Hearse and coworkers,¹³ the clinical reports of isolated myocytes in patients with tetralogy of Fallot,³⁴ as well as evidence that abrupt reoxygenation depletes endogenous antioxidants, in cyanotic patients¹⁶ even before aortic clamping is imposed. Hyperoxemic reoxygenation exaggerates the biochemical and structural adverse consequences of reintroduction of molecular oxygen in *in vivo* studies,¹³ whereas the damage is diminished by supplementation of the extracorporeal circuit primed with either antioxidants^{2, 7-9, 35} that limit the classic Haber-Weiss pathway of oxygen-mediated damage or substances that interfere with the more recently recognized L-arginine-nitric oxide (•NO) pathway.^{2, 36}

The aforementioned observations led to the cur-

rent study of normoxemic management, especially because the maximum velocity (V_{max}) for •NO synthase is Po₂-dependent and recent studies show that gradual reoxygenation is neuroprotective in chronically hypoxemic neuronal cells.²⁰ Our decision to proceed with a normoxemic strategy is in part based on recognition that hyperoxemic reoxygenation confers a negligible increase in oxygen content, because blood oxygen content is related primarily to hemoglobin content. Oxygen saturation is approximately 99% at Po₂ about 100 mm Hg so that potential cytotoxic effects of raising Po₂ to 400 mm Hg may overcome the almost imperceptible reduction in oxygen delivery of using a normoxemic approach. The hyperoxemic management method imposed during the latter phase of CPB and the post-CPB period and may have biased results unfavorably.

The failure to reduce damage by gradual reoxygenation *without* blood cardioplegia suggests that modification of Po₂ levels alone is an insufficient method of offsetting reoxygenation injury if hyperoxemia is allowed to occur. Normoxemia during

Antioxidant Reserve Capacity

Fig. 5. Myocardial antioxidant reserve capacity in control piglets undergoing hyperoxemic CPB and blood cardioplegia without hypoxemia (control) and hypoxemic piglets undergoing reoxygenation with either hyperoxemic (Po_2 400 mm Hg), or normoxemic (Po_2 100 mm Hg) blood cardioplegic solution (see text for description).

induction of CPB and blood cardioplegia was beneficial but did not avoid reoxygenation damage completely when followed by hyperoxemia; mild lipid peroxidation was evident and function recovered only 60% after the use of a cardioplegic strategy that allowed complete recovery in nonhypoxemic hearts despite hyperoxemia.

The effectiveness of normoxemic CPB with blood cardioplegia strategy was evident in the more extensive damage that followed gradual reoxygenation in the absence of surgical ischemia with blood cardioplegia. We ascribe the salutary effects of blood cardioplegia to (1) replenishment of amino acids depleted during hypoxemia,³⁷ (2) limited calcium loading during reoxygenation³⁸ because oxygen radical-induced cellular calcium overload and contractile abnormalities are reported recently in reoxygenated cardiac myocytes,^{38, 39} and (3) reduction in $\cdot\text{NO}$ overproduction, which can result in the toxic oxygen intermediate peroxynitrite ($\text{OONO}-$) that degrades to $\cdot\text{OH}$.⁴⁰ Further studies are needed to define the roles of the other

cardioplegic constituents in limiting reoxygenation injury as well as whether further reduction of Po_2 during initiation of CPB and before reoxygenation may provide added benefits when CPB and blood cardioplegia are used in the correction of congenital-causing cyanosis.

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