STUDIES OF HYPOXEMIC/ REOXYGENATION INJURY: WITHOUT AORTIC CLAMPING

IV. Role of the iron-catalyzed pathway: deferoxamine

This study tests the hypothesis that an iron chelator, deferoxamine, can reduce oxygen-mediated myocardial injury and avoid myocardial dysfunction after cardiopulmonary bypass by its action on the iron-catalyzed Haber-Weiss pathway. Twenty-one immature 2- to 3-week-old piglets were placed on cardiopulmonary bypass for 120 minutes, and five piglets served as biochemical controls without cardiopulmonary bypass. Five piglets underwent cardiopulmonary bypass without hypoxemia (cardiopulmonary bypass control). Sixteen others became hypoxemic while undergoing cardiopulmonary bypass for 60 minutes by lowering oxygen tension to about 25 mm Hg, followed by reoxygenation at oxygen tension about 400 mm Hg for 60 minutes. Oxygen delivery was maintained during hypoxemia by increasing cardiopulmonary bypass flow and hematocrit level. In seven piglets deferoxamine (50 mg/kg total dose) was given both intravenously just before reoxygenation and by a bolus injection (5 mg/kg) into the cardiopulmonary bypass circuit; nine others were not treated (no therapy). Myocardial function after cardiopulmonary bypass was evaluated from end-systolic elastance (conductance catheter) and Starling curve analysis. Myocardial conjugated diene production and creatine kinase leakage were assessed as biochemical markers of injury, and antioxidant reserve capacity was determined by measuring malondialdehyde in postcardiopulmonary bypass myocardium incubated in the oxidant, *t*-butylhydroperoxide. Cardiopulmonary bypass without hypoxemia caused no oxidant or functional damage. Conversely, reoxygenation (no therapy) raised myocardial conjugated diene levels and creatine kinase production (conjugated diene: 3.5 ± 0.7 absorbance 233 nm/min/100 g, creatine kinase: 8.5 ± 1.5 U/min/100 g; $p < 0.05$ versus cardiopulmonary bypass control), reduced antioxidant reserve capacity (malondialdehyde: 1115 ± 60 nmol/g protein at 4 mmol/L *t*-butylhydroperoxide; $p < 0.05$ versus control), and produced severe postbypass dysfunction (end-systolic elastance recovered only 39% ± 7%, $p < 0.05$ versus cardiopulmonary bypass control). Deferoxamine avoided conjugated diene production and creatine kinase release and retained normal antioxidant reserve, and functional recovery was complete (95% ± 11%, $p < 0.05$ versus no treatment). These findings show that iron-catalyzed oxidants may contribute to a reoxygenation injury and imply that deferoxamine may be used to surgical advantage. (J THORAC CARDIOVASC SURG 1995;110:1190-9)

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We have speculated that abrupt reoxygenation on cardiopulmonary bypass (CPB) causes an unintended oxygen-mediated myocardial injury that may add to subsequent intraoperative ischemic damage, resulting in postbypass myocardial dysfunction.[1] This concept of "iatrogenic reoxygenation injury on CPB" is supported by recent clinical observations from patients with cyanosis placed on CPB[2, 3] and from infants with hypoxemia placed on extracorporeal membrane oxygenation.[4] Our former studies[5] indicate that oxygen free radicals are in-

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volved in the pathogenesis of reoxygenation injury of hypoxemic immature hearts and that damage is reduced by catalase (a scavenger for hydrogen peroxide) and *N*-(2-mercaptopropionyl)-glycine, a scavenger for hydroxyl radical (·OH) and by the L-arginine analog that limits cytotoxic products of L-arginine-·NO-pathway.[5]

Iron may play a central role in oxygen radical generation and the subsequent chain reaction of lipid peroxidation, because ferric iron (Fe^{3+}) catalyzes the Haber-Weiss reaction, whereby O_2^- and H_2O_2 produce ·OH[6] that causes lipid peroxidation.[7, 8] Another potential deleterious role of iron is to catalyze the propagation of lipid peroxidation after its initiation by ·OH.[6]

Deferoxamine, a chelating agent with a high affinity for ferric iron (Fe^{3+}), has been found to be cardioprotective in various experimental models of ischemia/reperfusion[9-13] and has been used clinically to treat iron and aluminum overload.[14] This study tests the hypothesis that deferoxamine will limit production of iron-mediated oxidants and reduce reoxygenation injury of the hypoxemic immature heart.

Material and methods

Preparation. Twenty-six 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 for CPB, including vessel cannulation for blood sampling and monitoring, is similar to that described previously.[15]

Measurements

Acid-base and blood gas analyses. Systemic arterial and venous blood gas, electrolyte, and hemoglobin measurements were made every 15 minutes during CPB (Blood Gas System 288, CIBA-Corning, Medfield, Mass.). Body oxygen delivery and uptake were calculated by the following equations:

$$\text{Oxygen delivery } (Do_2, \text{ml/min/kg}) = F \times Cao_2/\text{body weight (kg)}$$

$$\text{Oxygen utilization } (Vo_2, \text{ml/min/kg}) = F \times (Cao_2\text{-}Cvo_2)/\text{body weight (kg)}$$

where F is pump flow (milliliters per minute), Cao_2 is oxygen content in arterial blood, and Cvo_2 is oxygen content in systemic venous blood.

Hemodynamics. Cardiac output was determined by duplicate injections of 1 ml of 4° C saline solution into a central venous catheter. Cardiac index (CI), systemic vascular resistance index (SVRI), and left ventricular stroke work index (LVSWI) were calculated with the following equations:

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

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

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

where MAP is mean aortic pressure, LAP is mean left atrial pressure, CVP is central venous pressure, HR is heart rate, and CO is cardiac output measured by thermodilution method or CPB pump flow rate in milliliters per minute.

Myocardial performance. LV pressure and conductance catheter signals were amplified and digitalized to inscribe left ventricular pressure-volume loops. After the procedure for correction of parallel conductance, a series of pressure-volume loops under variable loading conditions was generated by rapid transient occlusion of the inferior vena cava during a 7-second period of apnea, at a control condition, and 30 minutes after discontinuation of CPB.[16]

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), as described previously.[17] Postbypass LV contractility was assessed by percent recovery of prebypass control value (percent end-systolic elastance).

LV performance before and after CPB was also evaluated by infusing blood from the CPB circuit at 5 ml/min/kg over a 3-minute period while recording CO, MAP, and LAP to inscribe Starling function curves.

Myocardial conjugated diene and creatine kinase production. For blood biochemical assessments, the aorta was clamped temporarily (1 to 2 minutes) while the proximal aorta was perfused with blood by way of a calibrated pump to keep the aortic root pressure 50 to 60 mm Hg. Blood samples were withdrawn from the proximal aorta and the coronary effluent (coronary sinus blood) and analyzed for conjugated dienes (CDs) and creatine kinase (CK) during control (normoxemia), hypoxemia, and 5, 30, and 60 minutes of the reoxygenation period. They were centrifuged immediately for 5 minutes at 1000g, and plasma was then stored in liquid nitrogen and hydroxyconjugated diene levels determined as described by Lesnefsky and coworkers.[18] The plasma concentration of CK was measured by the method of Oliver.[19] Myocardial production of CDs and CK was calculated by the following equation:

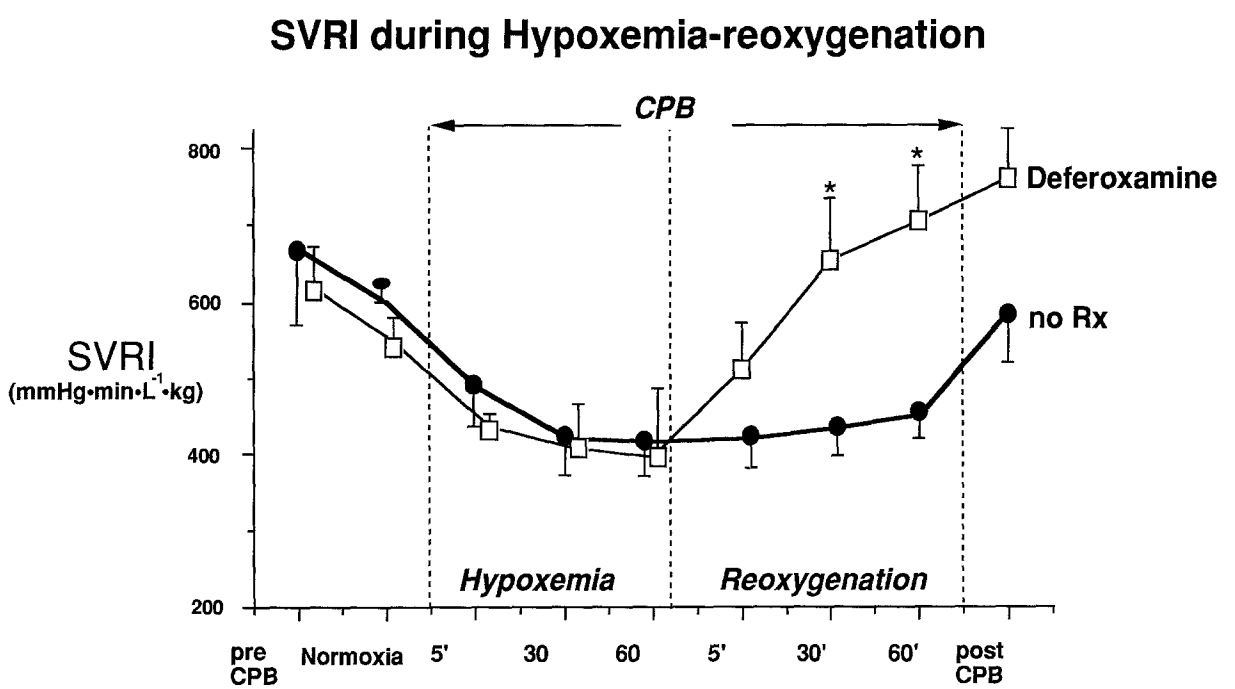

Fig. 1. Effects of hypoxemia-reoxygenation and administration of deferoxamine on systemic vascular resistance index *(SVRI)*. No Rx *(closed circles)*: reoxygenation on CPB without treatment ($n = 9$); deferoxamine *(open squares)*: piglets with deferoxamine administration 10 minutes before reoxygenation *(arrow)*. $^*p < 0.05$ versus no treatment.

Myocardial production = (Ccs-Ca) × CBF/(100 g)heart weight where Ccs and Ca are concentration in coronary sinus and arterial plasma blood sample (assuming plasma and red blood cell volumes are equal) and CBF (coronary blood flow, milliliters per minute) is a flow rate via a calibrated pump into the proximal aorta during temporary aortic clamping.

Antioxidant reserve capacity. The myocardial endogenous antioxidant state was assessed by determining in vitro lipid peroxidation in LV subendocardial muscle after each experiment was completed by the method of Godin as described previously.[20]

Experimental protocols

Controls. Five normoxemic, control piglets were anesthetized, instrumented, and observed over a 5-hour period to provide baseline functional and biochemical data without CPB. Five other piglets underwent 2 hours of CPB without hypoxemia (perfusion flow rate about 100 ml/min/kg) followed by 30 minutes of post-CPB observation while ventilated with 100% oxygen. This post-CPB oxygen management protocol was followed in all experimental studies.

Experimental. In 16 piglets CPB was started at normoxemia (oxygen tension [P_{O_2}] about 100 mm Hg). Mean aortic pressure was kept at 50 to 60 mm Hg by adjusting perfusion flow rate between 90 and 100 ml/min/kg. After 5 minutes of normoxemic perfusion, hypoxemia was produced on CPB by lowering inspired oxygen fraction (F_{IO_2}) in the oxygenator for 60 minutes by adding N_2 to the gas mixture to reduce P_{O_2} to about 25 mm Hg. Packed red blood cells were added to raise hematocrit level to 45% after hypoxemia was induced, and perfusion flow rate was increased to about 130 ml/min/kg. This hypoxemic interval was then followed by 60 minutes of reoxygenation while receiving CPB at P_{O_2} about 400 mm Hg.

NO TREATMENT. Nine piglets underwent aforementioned protocol without treatment.

DEFEROXAMINE TREATMENT. In seven piglets a bolus injection of 5 mg/kg was added to the CPB circuit 10 minutes before reoxygenation, at which time an intravenous infusion was started and continued until the end of CPB to provide an additional 45 mg/kg dose (total dose 50 mg/kg). This dose was selected because in two pilot experiments a total deferoxamine dose of 100 mg/kg resulted in severe vasoconstriction and precluded discontinuation of CPB (see "Results").

Final functional assessments were performed and specimens for biochemical analyses were obtained 30 minutes after CPB was discontinued.

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 experimen-

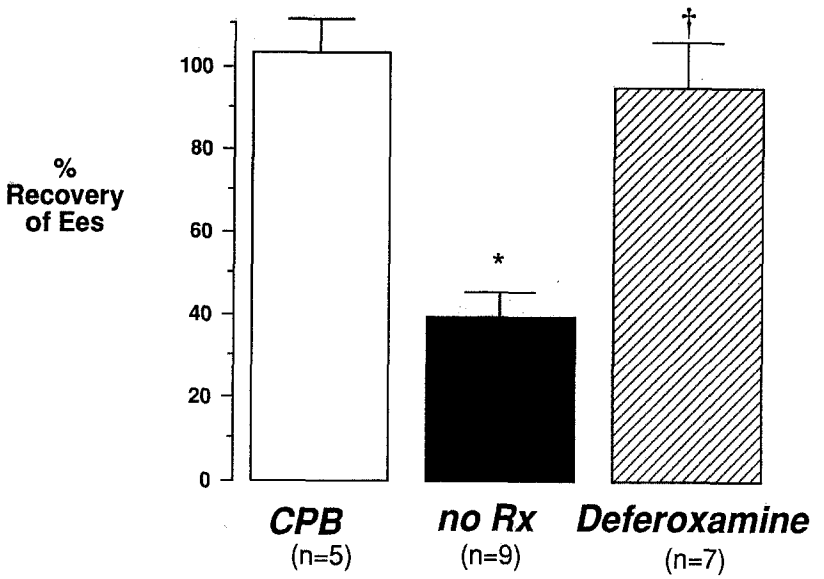

Fig. 2. Postbypass LV contractility. LV end-systolic elastance *(Ees)* after discontinuation of CPB, expressed as percentage of individual prehypoxemic values. *CPB,* Piglets underwent hyperoxic bypass without hypoxemia; *no Rx,* piglets untreated; *deferoxamine,* piglets treated with deferoxamine. *$p < 0.05$ versus CPB; †$p < 0.05$ versus no treatment.

tal groups. The relationship between functional impairment (percent elastance) 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

Hemodynamics. Systemic (CPB) flow averaged 100 ml/min/kg at about 60 mm Hg in control studies without hypoxemia to provide body oxygen delivery (Do_2) of 13 ± 0.3 ml/min/kg. A comparable body Do_2 was also achieved during hypoxemia by increasing CPB flow 30% and raising hematocrit level to 45%; no metabolic acidosis occurred because pH remained about 7.4 (Table).

Hypoxemia during CPB caused comparable systemic vasodilation in untreated and deferoxamine-treated groups as SVRI fell to 61% ± 5% and 63% ± 7%, respectively (Fig. 1). Deferoxamine raised SVRI progressively during reoxygenation, and resistance remained increased during the post-CPB interval; SVRI returned to control levels in untreated piglets (Fig. 1). The vasoconstrictive effect of deferoxamine was amplified in two pilot experiments in which a total 100 mg/kg hour dose raised SVRI 180% above pre-CPB values and precluded discontinuation of CPB.

LV performance. LV elastance returned to 102% ± 9% of control values after CPB without hypoxemia (Fig. 2). Conversely, reoxygenation without treatment was associated with only 39% ± 7% recovery of end-systolic elastance ($p < 0.05$ versus CPB). Deferoxamine treatment restored LV contractility to 95% ± 11% of pre-CPB levels ($p < 0.05$ versus no treatment) (Fig. 2). A parallel depression of myocardial performance occurred when functional reserve was tested by volume infusion (Starling curve analysis), because untreated piglets recovered only 38% ± 9% of LVSWI at LAP 12 mm Hg, whereas LVSWI recovered 84% ± 14% at comparable LAP in piglets treated with deferoxamine (Fig. 3).

Myocardial CD production. CD production was unchanged in piglets placed on CPB without hypoxemia (Fig. 4). Reoxygenation without treatment raised CD production 210% after 60 minutes of reoxygenation (3.46 ± 0.73 absorbance 233 nm/min/100 g, $p < 0.05$ versus CPB control), whereas negligible CD production occurred after deferoxamine treatment (CD production 0.7 ± 0.2, $p < 0.05$ versus no treatment).

CK release. Myocardial CK release was negligible in piglets placed on CPB without hypoxemia (Fig. 5). Reoxygenation without treatment caused a progressive release of CK after reoxygenation and

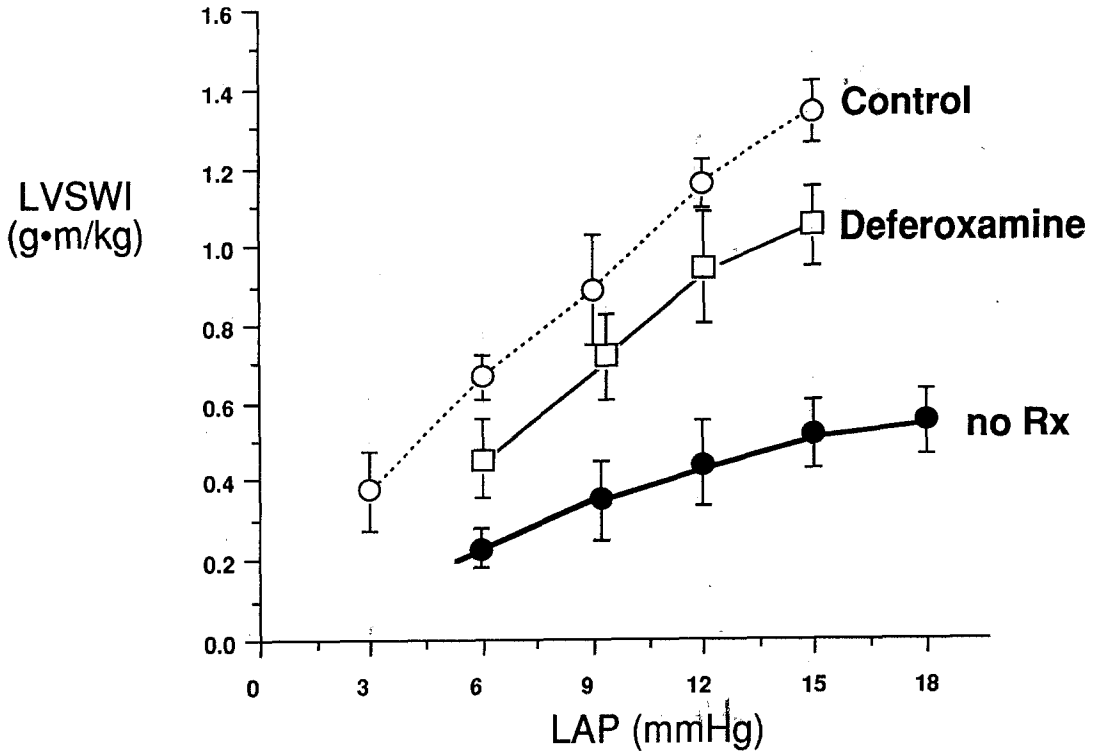

Fig. 3. LV performance by volume infusion before hypoxemia *(control: open circles)*, after reoxygenation with deferoxamine *(deferoxamine: open squares)*, and without treatment *(no Rx: closed circles)*. *LAP,* Left atrial pressure, *LVSWI,* left ventricular stroke work index.

reached 330% above prehypoxemic values 60 minutes after reoxygenation (8.5 ± 1.5 U/min/100 gm, $p < 0.05$ versus CPB control). In contrast, no CK production occurred in piglets receiving deferoxamine treatment.

Antioxidant reserve capacity. Reoxygenation (no treatment) reduced antioxidant reserve capacity as more malondialdehyde was produced in myocardial homogenates incubated with the oxidant *t*-butylhydroperoxide. Conversely, antioxidant reserve capacity remained normal when deferoxamine was administered before reoxygenation (Fig. 6).

Discussion

This study confirms an important role of iron-catalyzed oxidants in the pathogenesis of surgical reoxygenation injury of subjects at the onset of CPB by showing that the iron chelator, deferoxamine, reduces lipid peroxidation (CD production), membrane disruption (CK leakage), depletion of endogenous antioxidants, and depressed myocardial performance after reoxygenation.

The in vivo model of hypoxemia on CPB is a reproducible experimental setting that avoids hemodynamic deterioration and metabolic acidosis and ensures optimal systemic and myocardial oxygen supply. The use of increased perfusion flow rate and hematocrit level during hypoxemia provides comparable oxygen delivery to that occurring during normoxemia and avoided metabolic acidosis (Table). This compensated state has some relevance to chronic cyanosis, in which metabolic adaptations allow normal aerobic metabolism to prevail.[21] Functional and biochemical myocardial damage followed reoxygenation despite compensated hypoxemia. These observations are consistent with recent clinical findings of reoxygenation-induced lipid peroxidation and myocardial dysfunction in patients with hypoxemia placed on CPB or extracorporeal membrane oxygenation.[2, 4]

Among several oxygen radicals, hydroxyl radical (·OH) is the most toxic and causes lipid peroxidation and protein thiol oxidation,[7, 8] which results in myocardial and endothelial cell injury on reoxygenation/reperfusion.[22, 23] Iron may play a central role in oxygen radical generation and the subsequent chain

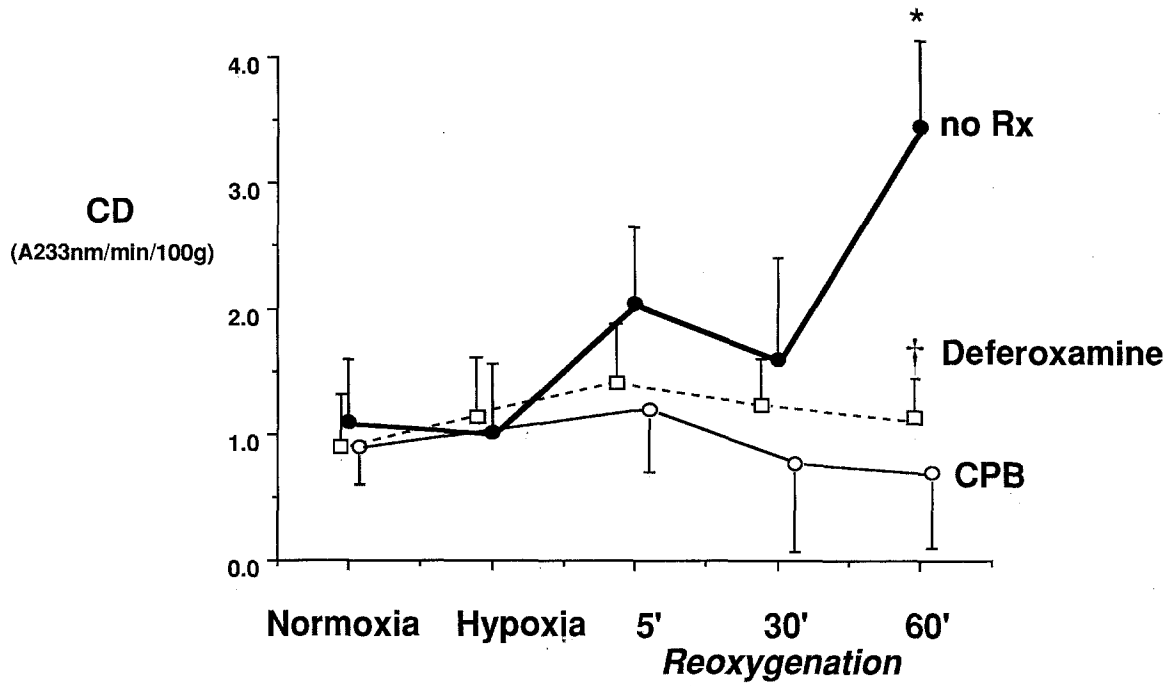

Fig. 4. Myocardial CD production during hypoxemia-reoxygenation on CPB. *CPB,* Piglets underwent hyperoxic bypass without hypoxemia, *no Rx,* piglets untreated; *deferoxamine,* piglets treated with deferoxamine. $^*p < 0.05$ versus CPB; $\dagger p < 0.05$ versus no treatment.

reaction of lipid peroxidation leading to membrane disruption. Ferric iron (Fe^{3+}) catalyzes the Haber-Weiss reaction, whereby O_2^- and H_2O_2 lead to the highly reactive ·OH radical.[6] Another potential detrimental role of iron is to catalyze the propagation of lipid peroxidation after its initiation by ·OH,[6] because lipid hydroperoxides formed in the course of ·OH-initiated lipid peroxidation can react with iron compounds to give alkoxyl and peroxyl radicals that further oxidize other fatty acids.[6] An alternate mechanism for generating ·OH may be the L-arginine-nitric oxide pathway, in which ·NO interacts with O_2^- to produce peroxynitrite ($OONO^-$), which further decays to ·OH.[24] A recent report documents that ·NO mediates iron release from ferritin,[25] and mobilized ferrous iron is thereby available to react with H_2O_2, suggesting the additional cytotoxic role of ·NO is in the iron-catalyzed Haber-Weiss reaction. O_2^- can also directly release "iron" from ferritin by reducing the metal to the Fe^{2+} state, thereby making it available for the aforementioned reactions.[26-28]

Deferoxamine is a chelating agent with a high affinity for ferric iron (Fe^{3+}). It enters cells readily,[11] prevents iron-catalyzed formation of ·OH from O_2^-,[29] and iron-dependent lipid peroxidation.[22] Its cardioprotective effect has been documented in experimental[9-12] and clinical[30, 31] ischemia/reperfusion injury studies by showing reduced free radical production by electron paramagnetic resonance spectroscopy,[11, 32] decreased oxygen-mediated lipid peroxidation,[13, 29] and improved myocardial function[9-12] with avoidance of high-energy phosphate depletion.[10, 13] Deferoxamine is known to scavenge O_2^-[33] and ·OH directly,[34] but its primary benefit appears related to its ability to bind iron, because some reports showed diminished efficiency in iron-loaded models.[9, 35]

Our observations in the hypoxemic/reoxygenation model showing that deferoxamine reduced lipid peroxidation, decreased enzyme leak, and improved recovery strengthen the concept that iron-mediated oxidants play a role in the pathogenesis of reoxygenation injury. Our dosage and administration protocol were derived from a previous in vivo study for regional ischemia[9] and was modified to account for the expanded volume introduced by CPB pump circuitry. The half-life of deferoxamine is short (about 5 minutes),[13] and thus the combination of a continuous intravenous infusion and a bolus injection seemed essential to attain therapeutic concentration. Progressive release of CK and CDs occurred

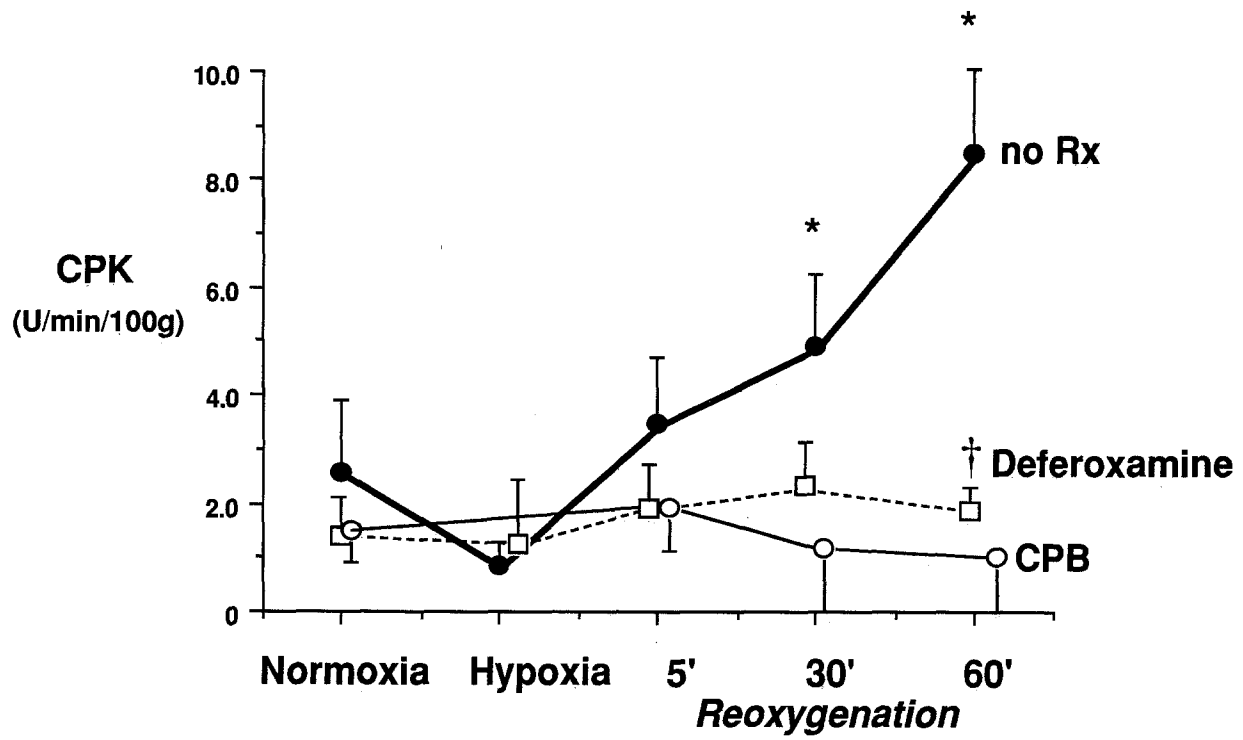

Fig. 5. Myocardial CK production during hypoxemia-reoxygenation on CPB. *CPB,* Piglets underwent hyperoxic bypass without hypoxemia, *no Rx,* piglets untreated; *deferoxamine,* piglets treated with deferoxamine. *$p < 0.05$ versus CPB; †$p < 0.05$ versus no treatment.

throughout 60 minutes of reoxygenation in untreated piglets, whereas sudden increases in myocardial and coronary sinus CD levels were reported in ischemia/reperfusion settings.[18, 36] This observation may indicate that ongoing oxidative damage may occur during reoxygenation and that an iron chelator should be administered for an extended interval after reoxygenation.

We observed that deferoxamine produced significant increase in SVRI in immature piglets, especially when a high dose was used (total 100 mg/kg), and this has not been reported previously. A similar vascular response occurred when L-arginine analogs (that block nitric oxide synthase) were added to the extracorporeal circuit prime in an effort to limit reoxygenation injury by interfering with the L-arginine-·NO pathway.[5] These systemic effects of deferoxamine, like those of L-arginine analogs, would not be detected in the isolated heart preparation and may reduce the physiologic effects of ·NO inhibition for modulating vascular tone and result in ischemia of organs that lack the vasoregulatory capacity of the myocardium (i.e., pancreas).[37] Recent reports suggest nitric oxide synthase, which contains iron, may be inhibited by other compounds that bind iron,[38] so that deferoxamine might cause vasoconstriction by inhibiting endothelium-derived relaxing factor production and may also thereby be cardioprotective by limiting NO production and preventing formation of $OONO^-$ that decomposes homolytically to produce ·OH.[24]

Implications. The concept that iron-mediated oxidant damage occurs during reoxygenation of subjects with cyanosis has potential therapeutic implications, because deferoxamine has been used to treat iron and aluminum poisoning[14] and a comparable dose to that used in this study has been shown to be safe in cardiac operations.[30] One prior report, however, noted acute toxicity after intravenous infusion of deferoxamine.[39] Clearly, the therapeutic dosage of deferoxamine must be defined by studying both systemic and myocardial effects before this drug could be recommended for clinical use.

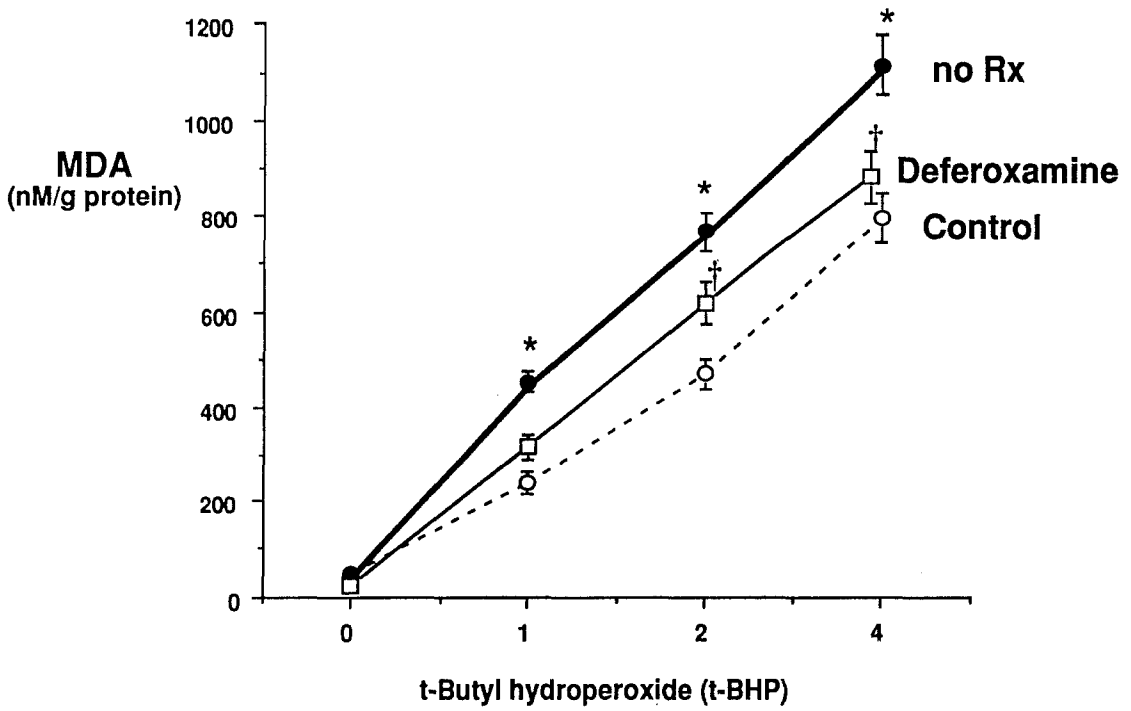

Fig. 6. Antioxidant reserve capacity. Vulnerability of reoxygenated myocardium to subsequent oxidant stress is determined by exposing tissue to 0 to 4 mmol/L *t*-butylhydroperoxide *(t-BHP)* for 30 minutes at 37° C with subsequent measurement of TBA-reactive substances. MDA standard curve is run simultaneously, as lipid peroxidation is expressed as MDA nmol/g protein. *Control,* Piglets without CPB; *no Rx,* piglets untreated; *deferoxamine,* piglets treated with deferoxamine. $*p < 0.05$ versus CPB, $\dagger p < 0.05$ versus no treatment. *MDA,* Malondialdehyde.

Table. *Physiologic profile during hypoxemia on CPB*

		Hypoxemia	
	Prehypoxemia	*No Rx*	*Deferoxamine*
pH	7.48 ± 0.02	7.41 ± 0.01	7.43 ± 0.02
Pa_{O_2} (mm Hg)	126.5 ± 14	24.0 ± 0.67*	23.2 ± 0.87*
Pa_{CO_2} (mm Hg)	25.1 ± 2.0	29.3 ± 1.5	29.9 ± 1.2
Hct (%)	31.5 ± 1.5	45.5 ± 1.4*	44.5 ± 2.4*
PFI (ml/min/kg)	109 ± 6	129.5 ± 5.9*	132.2 ± 4.3*
D_{O_2} (ml/min/kg)	13.0 ± 0.34	13.4 ± 0.89	13.9 ± 1.09
V_{O_2} (ml/min/kg)	7.4 ± 0.9	8.5 ± 0.5	7.9 ± 0.9

No Rx, No treatment; *Hct,* hematocrit on CPB; *PFI,* perfusion flow rate on CPB (ml/min/kg); Do_2, oxygen delivery (ml/min/kg); Vo_2, oxygen consumption (ml/min/kg).

$*p < 0.05$ versus prehypoxemic values (paired *t* test).

We conclude that deferoxamine administered intravenously before and during reoxygenation, coupled with adding a bolus injection to the extracorporeal circuit, reduces oxygen-mediated myocardial damage substantially by limiting lipid peroxidation, enzymatic damage, and depressed myocardial performance. These findings support an important role of iron-catalyzed oxidants in the pathogenesis of reoxygenation injury. However, the observed production of systemic vasoconstriction in a dose-dependent fashion might nullify its cardioprotective action. Additional studies are needed to define the

safe therapeutic dosage before consideration of use of this drug during surgical correction of congenital defect causing cyanosis in infants.

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