SIALYL LEWIS^X OLIGOSACCHARIDE PRESERVES CARDIOPULMONARY AND ENDOTHELIAL FUNCTION AFTER HYPOTHERMIC CIRCULATORY ARREST IN LAMBS

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Objective: Neutrophil adhesion to endothelium contributes to cardiopulmonary dysfunction after cardiac surgical procedures. Initial neutrophil-endothelial interactions involve selectins, which bind carbohydrate ligands, such as sialyl-Lewis^X. Blockade of selectin-mediated neutrophil interactions with CY1503, a synthetic oligosaccharide analog of sialyl-Lewis^X, could limit neutrophil-mediated injury after cardiopulmonary bypass.

Methods: The efficacy of CY1503 treatment was tested in a lamb model of cardiopulmonary bypass with hypothermic circulatory arrest. Neonatal lambs received CY1503 (n = 6, CPB-CY1503) or saline solution vehicle (n = 7, CPB-saline) into the pump prime before bypass and as a continuous infusion throughout reperfusion. Five lambs served as control animals for in vitro microvessel studies. Indexes of myocardial function (preload recruitable stroke work index, and rate of pressure rise) and pulmonary function (compliance, airway resistance, and arterial Po₂) were measured before bypass and during reperfusion. The effect of CY1503 on endothelium-dependent vascular reactivity was assessed by means of in vitro pulmonary and coronary microvessel studies.

Results: Myocardial function was depressed after circulatory arrest, but CY1503 preserved function near baseline (36% ± 25% vs 99% ± 19% of baseline at 3 hours of reperfusion). CY1503-treated animals also demonstrated improved pulmonary function during reperfusion. In vitro microvessel analysis of vascular reactivity revealed endothelial dysfunction after circulatory arrest compared with control lambs. CY1503-treated lambs (CPB-CY1503) had intact endothelial function, as demonstrated by normal vasodilatory responses to endothelium-dependent vasodilators.

Conclusions: CY1503 preserves cardiopulmonary and endothelial function after cardiopulmonary bypass and hypothermic circulatory arrest in neonatal lambs. This suggests a role for selectin-mediated, neutrophil-endothelial interactions in the inflammatory response after cardiac operations. (*J Thorac Cardiovasc Surg* 2000;120:230-7)

Most reparative cardiac surgical procedures require cardiopulmonary bypass (CPB), which results in an obligatory period of myocardial and pulmonary

ischemia with subsequent reperfusion.¹ Furthermore, neonatal cardiac operations often necessitate deep hypothermic circulatory arrest (DHCA), which results

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in whole body ischemia-reperfusion. These planned periods of ischemia-reperfusion can initiate tissue and vascular injury throughout the body, most notably in the heart and lungs.² Activation of inflammatory cascades by the extracorporeal circuit may also contribute to inflammatory injury after CPB.³

Interactions between leukocytes and vascular endothelium are integral to various inflammatory disorders, including ischemia-reperfusion injury. The inflammatory events after cardiopulmonary support are known to be neutrophil mediated.³ Correspondingly, expression of a variety of leukocyte-endothelial adhesion molecules is upregulated after CPB.^{4,5} Neutrophil-endothelial interactions are a multistep process whereby adhesion molecules on both leukocytes and vascular endothelium mediate the initial rolling and subsequent firm adhesion of leukocytes to endothelial cells.⁶ The initial rolling of leukocytes along the endothelial surface is mediated by the selectins (P-, E-, and L-selectin), which recognize fucosylated carbohydrate ligands on the neutrophil, such as sialyl Lewis^x antigen. The rolling of leukocytes, which occurs at the onset of inflammation, is a prerequisite for firm leukocyte adhesion and transendothelial leukocyte migration.⁶

Prevention of leukocyte adhesion to the endothelium may be a potential strategy to reduce the inflammation-related morbidity of CPB by preventing reperfusion injury to the heart, lungs, and other organs. Inhibition of the initial selectin-mediated leukocyte-endothelial interactions is intuitively attractive. In blood-perfused isolated lamb hearts subjected to 2 hours of hypothermic ischemia followed by reperfusion, we have demonstrated preservation of myocardial contractile function by selectin blockade with either fucoidin⁷ or the synthetic sialyl Lewis^x analog CY1503 (unpublished data). In other animal models of inflammation or ischemic injury, administration of CY1503 has been shown to be effective in reducing neutrophil accumulation and tissue injury, including myocardial infarction,⁸⁻¹¹ acute lung injury,¹²⁻¹⁴ trauma,¹⁵ transplantation,^{12,16} and septic shock.¹³ Animal models of myocardial infarction have demonstrated that CY1503 treatment before reperfusion reduces infarct size, preserves contractile function, and maintains normal coronary vascular reactivity.⁸⁻¹⁰ In a similar manner we reasoned that administration of CY1503 might limit the inflammatory tissue injury related to CPB with DHCA (CPB-DHCA). Because circulatory support is commonly associated with alterations in myocardial performance, pulmonary function, and vascular reactivity postoperatively, the

present study was undertaken to determine whether administration of CY1503 could preserve myocardial, pulmonary, and vascular endothelial function in a neonatal lamb model of CPB and circulatory arrest.

Material and methods

Experimental preparation. Neonatal lambs (age, 5-7 days; weight, 5-8 kg) were anesthetized with intramuscular ketamine (40 mg/kg) and intubated. Mechanical ventilation was instituted with a Servo 900C ventilator (Siemens, Danvers, Mass) with tidal volumes of 15 mL/kg, a fraction of inspired oxygen of 1.0, and 3 cm H₂O positive end-expiratory pressure. The respiratory rate was adjusted to maintain PCO₂ between 35 and 45 mm Hg. After venous access had been established, an induction dose of fentanyl (300 µg/kg) was given, and continuous infusions of fentanyl (50 µg · kg⁻¹ · h⁻¹), ketamine (5 mg · kg⁻¹ · h⁻¹), and midazolam (0.5 mg · kg⁻¹ · h⁻¹) were begun and given throughout the remainder of the experiment, excluding the circulatory arrest period. Catheters were placed in the femoral artery and vein, the left atrium, and the main pulmonary artery for hemodynamic measurements and blood sampling. Sonomicrometry crystals were placed on the epicardium of the left ventricular free wall along the equatorial circumference for measurement of myocardial segmental shortening.¹⁷ After systemic heparinization (400 U/kg), a 5F vascular sheath was inserted into the left ventricle through the apex for pressure measurement with a pressure transducer (SPC-350; Millar Instruments, Inc, Houston, Tex) and for venting during CPB. The extracorporeal bypass circuit consisted of a standard roller pump (Cardiovascular Instruments, Wakefield, Mass), sterile new membrane oxygenator (VPCML, Cobe Laboratories Inc, Lakewood, Colo), and sterile new tubing. The pump prime consisted of 200 mL of Normosol solution and 500 mL of homologous donor blood (hematocrit value, ~20%) warmed to 37°C.

Experimental procedure. After baseline ventricular and pulmonary function measurements had been recorded, CPB was instituted at 150 mL · kg⁻¹ · min⁻¹ through an 8F arterial cannula placed in the right femoral artery and a 24F venous cannula placed in the right atrium. After 5 minutes of normothermic CPB, phentolamine hydrochloride (0.2 mg/kg) was given, systemic cooling was begun, and the esophageal temperature was lowered to 15°C over 25 minutes by the pH-stat blood gas strategy. A 2-hour period of hypothermic circulatory arrest followed, with additional topical cooling of the heart with saline solution at 4°C. CPB was reinitiated after 2 hours of circulatory arrest, and the animals were rewarmed to an esophageal temperature of 35°C over 30 minutes. Spontaneous return of sinus rhythm occurred in all animals, thus allowing the animals to be separated from CPB after the 30-minute rewarming period. The animals' lungs were not ventilated during CPB or circulatory arrest. During the subsequent 3-hour reperfusion period after termination of CPB, the animals' lungs were ventilated and the animals did

not receive inotropic support. Myocardial and pulmonary function measurements were repeated at 1, 2, and 3 hours of reperfusion.

Myocardial function: Preload recruitable stroke work index. Preload recruitable stroke work index (PRSWI), a load-independent measure of cardiac function, was measured during transient inferior vena caval occlusion.¹⁷ Left ventricular pressure (Millar transducer) and segment length (sonomicrometry crystals) were measured simultaneously during successive cardiac cycles to generate pressure-segment length loops; each loop represents the regional myocardial stroke work of a specific cardiac cycle. During caval occlusion, a series of pressure-segment length loops were generated as preload decreased decrementally. Regional myocardial stroke work is linearly related to end-diastolic segment length, and the slope of the regression of this relationship determines the PRSWI. PRSW was determined at baseline (before hypothermic circulatory arrest) and at 1, 2, and 3 hours of reperfusion. Each determination represented an average of 3 caval occlusions, and regressions were excellent in all animals ($r^2 \geq 0.98$).

Pulmonary function. The lambs' lungs were ventilated with a Servo 900C ventilator (Siemens) with tidal volumes of 15 mL/kg, a fraction of inspired oxygen of 1.0, and 3 cm H₂O positive end-expiratory pressure. To set a consistent lung volume history, lungs were inflated to a sustained static inflation pressure of 40 cm H₂O for 30 seconds immediately before measurements. Pulmonary mechanics were measured with the integrated pulmonary mechanics module for the Servo 900C ventilator (Siemens). Dynamic pulmonary compliance was determined as expired tidal volume divided by change in pressure (end-inspiratory pressure – positive end-expiratory pressure). Passive expiratory airway resistance was determined as change in pressure (end-inspiratory pressure – positive end-expiratory pressure) divided by peak expiratory flow. Pulmonary function and simultaneous arterial blood gas measurements were made at baseline (before CPB) and 1, 2, and 3 hours of reperfusion.

Edema formation. Samples of the left ventricle, lung, kidney, and liver were harvested after 3 hours of reperfusion. Tissue samples were weighed, desiccated in an oven for 48 hours, and reweighed. Wet/dry weight ratios were calculated and expressed as means $\pm$ SD.

In vitro microvessel studies. Pulmonary arterioles (75–200 μ m) were dissected from the lungs of sheep by means of a 10–60 $\times$ dissecting microscope (Olympus Optical Co, Ltd, Tokyo, Japan). In a similar manner, coronary arterial microvessels (75–200 μ m in internal diameter) were dissected from the subepicardial region of the left ventricle of each heart. Tissue samples were immediately placed in cold ($\sim 4^\circ\text{C}$) Krebs buffer solution. Microvessels were placed in a Plexiglas organ chamber (Rohm and Haas Company, Philadelphia, Pa), cannulated with dual glass micropipettes measuring 30 to 80 μ m in diameter, and secured with 10-0 nylon monofilament suture (Ethicon, Inc, Somerville, NJ). 3-(N-morpholino) propanesulfonic acid (MOPS) buffer solution warmed to 37°C was continuously circulated through the

organ chamber and a reservoir (total fluid volume, 100 mL). Pulmonary microvessels were pressurized to 20 mm Hg, and coronary microvessels were pressurized to 40 mm Hg in a no-flow state by using a burette manometer filled with MOPS buffer solution. MOPS buffer solution was composed of the following: NaCl, 145.0 mmol/L; KCl, 4.7 mmol/L; CaCl₂, 2.0 mmol/L; MgSO₄, 1.2 mmol/L; glucose, 5.0 mmol/L; pyruvate, 2.0 mmol/L; ethylenediaminetetraacetic acid, 0.02 mmol/L; NaH₂PO₄, 1.2 mmol/L; and MOPS, 3.0 mmol/L.

An inverted microscope (40–200 $\times$; Olympus) was connected to a video camera, and the vessel image was projected onto a black-and-white television monitor (Hitachi Ltd, Tokyo, Japan). An electronic dimension analyzer (Living System Instrumentation, Burlington, Vt) was used to measure the internal lumen diameter. Measurements were recorded with a strip-chart recorder. Vessels were allowed to equilibrate for at least 30 minutes in Krebs buffer solution before a drug intervention.

Relaxation responses of pulmonary and coronary microvessels were examined after precontraction of vessels by 20% to 60% with the thromboxane A₂ analog U46619. Once the steady-state tone was attained, the dose responses to acetylcholine (10^{-9} – 10^{-4} mol/L) and sodium nitroprusside (10^{-9} – 10^{-4} mol/L) were examined in pulmonary vessels, and the responses to the calcium ionophore A23187 (10^{-9} – 10^{-5} mol/L) and sodium nitroprusside (10^{-9} – 10^{-4} mol/L) were examined in coronary vessels. Acetylcholine was observed to cause contraction of sheep coronary microvessels and was therefore not used to assess endothelium-dependent relaxation. Drugs were applied extraluminally. Measurements were always taken 2 to 3 minutes after the drug was administered, when the response had stabilized. The vessels were washed 3 times with MOPS buffer solution and allowed to equilibrate in a drug-free buffer solution for 10 to 15 minutes between pharmacologic interventions.

Drugs. Acetylcholine chloride, A23187, U46619, MOPS, and sodium nitroprusside were obtained from Sigma Chemical Company (St Louis, Mo). U46619 was dissolved in ethanol and water. Other drugs were dissolved in ultrapure distilled water. All solutions were prepared on the day of the study.

Experimental groups. The sialyl Lewis^x analog (CY1503) used in this study was provided by Cytel Corporation (San Diego, Calif). Animals in the experimental group (CPB-CY1503, $n = 6$) received CY1503 (20 mg/kg) added to the pump prime before institution of CPB and a continuous infusion ($3.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) during reperfusion to attain constant levels of CY1503 before ischemia and during the entire period of reperfusion. Control animals (CPB-saline, $n = 7$) received saline vehicle alone. Control animals for in vitro microvessel studies were anesthetized, intubated, ventilated, and received a sham sternotomy, after which peripheral segments of lung and myocardial samples for microvessel analysis were harvested.

Data analysis. Results from myocardial and pulmonary function measurements are expressed as percentage recovery of baseline (mean $\pm$ SD). Differences between groups were determined by repeated-measures analysis of variance.

Fig 1. Myocardial contractile function in control and CY1503-treated animals during reperfusion. The *upper panel* demonstrates that PRSWI (a load-independent measure of contractile function) is greater in Cy1503-treated animals throughout reperfusion ($P = .001$, overall difference between groups determined by repeated-measures analysis of variance). The *lower panels* show that +dP/dt and -dP/dt (load-dependent measures of myocardial function) are greater in CY1503-treated animals throughout reperfusion ($P = .04$ and $.01$, respectively). Results are expressed as the percentage recovery of baseline value (mean $\pm$ SD).

The response of microvessels to each agent or pressure intervention was examined once in each animal. Dose-response data from each experimental group were pooled. Differences between groups were determined by using 2-factor repeated-measures analysis of variance and the Scheffé test post hoc. Values are expressed as means $\pm$ SEM.

Animals in this study 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 and published by the National Institutes of Health (National Institutes of Health publication No. 85-23, revised in 1985).

Fig 2. Pulmonary function in control and CY1503-treated animals during reperfusion. Although pulmonary mechanics are not markedly impaired after CBP-DHCA, dynamic lung compliance and expiratory airway resistance are slightly better in CY1503-treated animals throughout reperfusion ($P = .014$ and $.037$, respectively; overall difference between groups determined by repeated-measures analysis of variance). Results are expressed as the percentage recovery of baseline value (mean $\pm$ SD).

Table I. Baseline myocardial and pulmonary function measurements

	CPB-saline	CPB-CY1503
PRSWI ($\text{erg} \cdot 10^3/\text{cm}^3$)	57 $\pm$ 11	69 $\pm$ 26
+dP/dt (mm Hg/s)	1012 $\pm$ 287	922 $\pm$ 350
-dP/dt (mm Hg/s)	892 $\pm$ 311	735 $\pm$ 391
Dynamic pulmonary compliance (mL/cm H ₂ O)	8.2 $\pm$ 2.3	9.9 $\pm$ 3.1
Expiratory airway resistance (cm H ₂ O $\cdot$ L ⁻¹ $\cdot$ s ⁻¹)	22.9 $\pm$ 4.1	23.2 $\pm$ 8.0

Data are presented as means $\pm$ SD.

Results

Myocardial function and hemodynamics. There were no baseline differences in the measured indices of myocardial function between the two groups (Table I). Fig 1 displays the percentage recovery of myocardial function (PRSWI and rate of pressure rise [+dP/dt] and fall [-dP/dt]) at 1, 2, and 3 hours' reperfusion after CPB-DHCA. Myocardial function was markedly depressed throughout the 3-hour reperfusion period in untreated lambs subjected to 2 hours of DHCA. Myocardial func-

Table II. Hemodynamics and Pao_2

	CPB-saline				CPB-CY 1503			
	Pre-CPB baseline	One-hour reperfusion	Two-hour reperfusion	Three-hour reperfusion	Pre-CPB	One-hour reperfusion	Two-hour reperfusion	Three-hour reperfusion
SBP (mm Hg)	63 ± 15	43 ± 14	52 ± 16	48 ± 12	52 ± 19	52 ± 12	63 ± 16	61 ± 12*
MAP (mm Hg)	52 ± 10	28 ± 7	38 ± 12	34 ± 10	44 ± 16	35 ± 8	42 ± 14	46 ± 10
PAP (mm Hg)	20 ± 7	19 ± 8	27 ± 15	22 ± 7	15 ± 4	18 ± 6	22 ± 6	22 ± 4
LAP (mm Hg)	2 ± 2	4 ± 4	5 ± 3	3 ± 3	2 ± 2	4 ± 3	4 ± 3	4 ± 3
Pao_2	585 ± 61	474 ± 161	450 ± 172	356 ± 161	509 ± 89	552 ± 108	571 ± 84	543 ± 72*

Data are presented as means ± SD. SBP, Systolic blood pressure; MAP, mean arterial pressure; PAP, mean pulmonary arterial pressure; LAP, left atrial pressure.

* $P < .05$ versus the CPB-saline group, as determined by repeated-measures analysis of variance.

tion was significantly preserved in CY1503-treated animals; recovery of PRSWI, $+dP/dt$, and $-dP/dt$ was better in the CY1503-treated lambs than in the untreated control animals ($P = .001$, $.04$, and $.01$, respectively). The improvement in myocardial function corresponded to greater systolic and mean arterial pressure in CY1503-treated animals, with no difference in left atrial pressure between groups (Table II).

Pulmonary function. Pulmonary mechanics were not significantly impaired after CBP-DHCA (Fig 2); dynamic lung compliance and airways resistance approached baseline values in untreated animals during reperfusion. In spite of this, animals treated with CY1503 exhibited slightly better dynamic lung compliance and expiratory airway resistance than did untreated animals (repeated-measures analysis of variance revealed overall differences between groups: $P = .014$ and $.037$, respectively). Pulmonary gas exchange was mildly impaired in the untreated lambs after CPB, as demonstrated by the progressive fall in arterial PO_2 (Pao_2) during reperfusion. Gas exchange was better preserved in CY1503-treated animals, as demonstrated by higher Pao_2 throughout reperfusion (Table II).

Wet/dry weight ratio. No differences were noted in wet/dry weight ratios of heart, lung, liver, or kidney between the untreated and CY1503-treated groups (Table III).

Vessel characteristics. Pulmonary microvessels averaged $160 \pm 9 \mu m$, $149 \pm 10 \mu m$, and $160 \pm 10 \mu m$ in the control, CPB-saline, and CPB-CY1503 groups, respectively. The percentage of precontraction after application of U46619 was $45\% \pm 6\%$, $47\% \pm 5\%$, and $45\% \pm 3\%$ in the control, CPB-saline, and CPB-1503 groups, respectively. Coronary microvessels averaged $181 \pm 7 \mu m$, $164 \pm 9 \mu m$, and $186 \pm 6 \mu m$ in the control, CPB-saline, and CPB-CY1503 groups, respectively. The percentage of precontraction after application of U46619 was $38\% \pm 2\%$, $42\% \pm 2\%$, and $45\% \pm 4\%$

in the control, CPB-saline, and CPB-CY1503 groups, respectively.

Pulmonary vascular responses. Pulmonary endothelium-dependent microvascular relaxation to acetylcholine was impaired after CPB compared with the control response, whereas vessels from CY1503-treated animals showed normal relaxation to acetylcholine (Fig 3, A). Endothelium-independent relaxation to sodium nitroprusside was similar in all groups (Fig 3, B).

Coronary vascular responses. Coronary endothelium-dependent microvascular relaxation to the calcium ionophore A23187 was reduced after CPB compared with the control response, whereas vessels from CY1503-treated animals showed normal relaxation to A23187 (Fig 4, A). Endothelium-independent relaxation to sodium nitroprusside was similar in all groups (Fig 4, B).

Discussion

Ischemia-reperfusion injury is a complex process that occurs in multiple pathologic states, including cardiovascular operations, transplantation, trauma, stroke, and myocardial infarction.² CPB is a particularly potent stimulus for inflammatory injury; activation of blood components by the extracorporeal circuit may augment tissue reperfusion injury.^{1,3} The inflammatory process resulting from ischemia with subsequent reperfusion involves (1) neutrophil recruitment and activation and (2) release of proinflammatory cytokines, toxic oxygen metabolites, and proteases.⁶ Interactions between neutrophils and endothelium are integral to the deleterious inflammatory process.⁶ The initial tethering of neutrophils to endothelium is mediated by the selectins, glycoprotein endothelial adhesion molecules that bind to oligosaccharide ligands on neutrophils, including sialyl Lewis^x.¹⁸ P-selectin is stored preformed in endothelial Weibel-Palade bodies, and surface expression can occur within minutes in response to

Fig 3. In vitro dose responses of pulmonary microvessels to the endothelium-dependent and endothelium-independent agonists acetylcholine (**A**) and sodium nitroprusside (**B**) in control, CPB-saline, and CPB-CY1503 groups (displayed as log to the base 10 of the specific vasodilator). Vessels were precontracted with U46619, and responses are given as percentage relaxation of U46619-induced vessel contraction. CPB with circulatory arrest resulted in significant pulmonary endothelial dysfunction, which was prevented by CY1503 treatment. Asterisks indicate overall difference in dose response of the control and CPB-CY1503 groups compared with the CPB-saline group, as determined by repeated-measures analysis of variance and the Scheffé test post hoc ($P < .01$).

histamine, thrombin, complement, and hypoxia. By contrast, E-selectin is synthesized de novo in response to cytokines, such as interleukin 8, interleukin 1, lipopolysaccharide, and tumor necrosis factor α ; maximal expression occurs within 4 to 6 hours and persists for 24 to 48 hours. After the initial step of selectin-mediated leukocyte rolling, firm adhesion between neutrophils and endothelial cells occurs through interaction of integrins and intercellular adhesion molecules, bringing activated neutrophils into intimate contact with the endothelial surface to allow local damage to endothelium and transendothelial migration into parenchymal tissue.⁶

Interruption of neutrophil-endothelial adhesion offers the potential to abort or diminish the inflammatory process with obvious therapeutic potential. Antibodies to various adhesion molecules have protective effects after myocardial ischemia-reperfusion. Monoclonal antibodies to CD11 and CD18 reduce myocardial and endothelial damage after global or focal ischemia.¹⁹⁻²¹ Monoclonal antibodies directed against P-selectin,²² L-selectin,²³ and intercellular adhesion molecule 1²⁴ have been shown to improve recovery of contractile and endothelial function and decrease neutrophil accumulation after temporary coronary ligation in feline or canine hearts.

We reasoned that blockade of selectin-mediated neutrophil-endothelial interactions could prevent inflammatory tissue injury after CPB and hypothermic

Table III. Wet/dry weight ratios

	Heart	Lung	Kidney	Liver
CPB-saline	1.38 $\pm$ 0.30	2.42 $\pm$ 0.89	2.75 $\pm$ 0.86	2.05 $\pm$ 0.77
CPB-CY1503	1.41 $\pm$ 0.26	1.84 $\pm$ 0.67	2.05 $\pm$ 0.45	1.66 $\pm$ 0.31

Data are presented as means $\pm$ SD.

circulatory arrest. CY1503, a synthetic oligosaccharide analog of sialyl Lewis^x, is thought to reduce neutrophil adherence to endothelium by saturation blockade of selectins. CY1503 has been shown to reduce tissue injury in multiple animal models of ischemia-reperfusion,⁸⁻¹⁶ and the present data provide further evidence of the protective effects of CY1503 in another animal model. Pulmonary and coronary endothelial dysfunction, as normally accompanies CPB, was absent in CY1503-treated lambs subjected to CPB and circulatory arrest. This finding correlated with significant preservation of myocardial contractile and pulmonary function in the CPB-CY1503 group. Although the data suggest that preservation of myocardial and pulmonary function results from selectin blockade, other potential mechanisms of protection cannot be ruled out. The data are consistent with observations made in CY1503-treated pigs undergoing CPB,²⁵ but cardiopulmonary and endothelial preservation associated with CY1503 treatment was more dramatic in the neonatal lambs we studied. The greater efficacy of

Fig 4. In vitro dose-responses of coronary microvessels to the endothelium-dependent and endothelium-independent agonists A23187 (**A**) and sodium nitroprusside (**B**) in the control, CPB-saline, and CPB-CY1503 groups (displayed as log to the base 10 of the specific vasodilator). Vessels were precontracted with U46619, and responses are given as percentage relaxation of U46619-induced vessel contraction. CPB with circulatory arrest resulted in significant coronary endothelial dysfunction, which was prevented by CY1503 treatment. Asterisks indicate overall difference in dose response of the control and CPB-CY1503 groups compared with the CPB-saline group, as determined by repeated-measures analysis of variance and the Scheffé test post hoc ($P < .01$).

CY1503 in neonatal lambs may be an age- or species-dependent effect. Alternatively, CY1503 was given in the present study before CPB-DHCA and continuously during reperfusion, whereas in the previous study it was given as a 1-time bolus before aortic crossclamp removal. It seems likely that microvascular distribution of CY1503 is more homogeneous when given before ischemia, and maintaining continuous levels of the oligosaccharide during reperfusion may also be necessary for optimal efficacy. Our findings are also consistent with those of multiple animal myocardial infarction studies, which have demonstrated that CY1503 treatment before reperfusion reduces infarct size and neutrophil accumulation while improving cardiac contractility and endothelium-dependent vasodilation.⁸⁻¹¹ Similarly, CY1503 has also been shown to reduce tissue injury in animal models of acute lung injury,¹²⁻¹⁴ trauma,¹⁵ transplantation,^{12,16} and septic shock.¹³

Preservation of endothelial function by CY1503 is a significant observation of this study. The endothelium appears to be an early target of the reperfusion-related inflammatory process, and endothelial dysfunction occurs early after ischemia-reperfusion.^{10,26,27} Regional and global myocardial ischemia-reperfusion impairs endothelium-dependent vasoreactivity and increases capillary permeability.^{10,22,28} Endothelial injury during reperfusion may precede damage to the myocardium, and interventions that preserve endothelial function have been associated with improved

myocardial contractile function.^{28,29} It is unclear in these studies whether preservation of endothelial function is causative or correlative, but the results of Tofukuji and colleagues²⁵ might suggest the latter. Endothelial dysfunction and concomitant alterations in vascular reactivity are widely known sequelae of CPB,^{26,30} and preservation of endothelial function in lambs receiving CY1503 is thus notable.

In conclusion, the present study demonstrates the potential utility of antiadhesion therapy in neonatal animals subjected to CPB with DHCA. Our findings suggest that selectin-mediated neutrophil-endothelial interactions play an important role in cardiopulmonary and endothelial dysfunction after DHCA in neonatal lambs. Blockade of the inflammatory process by agents that interfere with leukocyte-endothelial interactions may help optimize organ preservation during cardiac operations.

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